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VOL. XIII ST. LOUIS, MO., MARCH, 1928 NO. 6

CLINICAL AND EXPERIMENTAL

AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS

THE RELATION OF CLINICAL PATHOLOGY TO PRECLINICAL MEDICINE*

BY DR. WM. G. EXTON, NEWARK, N. J.

MANY years ago when I was doing general practice, I had to listen so often and so long to what folks had to say about their babies and their children that I vowed that if ever I had any, I would never talk about mine. Well, when I got older I did have some children, but as mine were really just a little bit different from other folks', I was never quite able to keep that vow.

Again, it has been my good fortune to enjoy many a dinner with pleasant company around me only to have the effect somewhat neutralized by a so-called "presidental address." Here, too, I made resolutions, and likewise find it impossible to keep them as perfectly as I want.

As I get older I find myself more and more agreeing with Phillips Brooks, who once defined life as a series of compromises, and if you will permit it, I will compromise with you by not inflicting on you a real presidential address but rather running through a few thoughts which have occurred to me from time to time in connection with the latest development in the practice of medicine which is sometimes spoken of as "health examinations," but which I prefer to designate by some such term as "preclinical medicine." I think this movement is a most important one, which had to wait until medical science had advanced to a certain point before it was worth while putting into practice, although the idea is not at all new.

One of our honored guests has asked me to define "clinical pathologist." May I say that to my mind clinical pathology represents an array of facts and procedures, the offsprings of scientific research, and that the clinical pathologist is a specialist who bridges two gulfs: one between the research laboratory and the practitioner, and one between the physician or surgeon and the patient. New facts and methods are constantly being brought to

*Presidential address at the Sixth Annual Meeting of the American Society of Clinical Pathologists, Washington, D. C., May 14, 1927.

light which offer the physician and surgeon a truer insight into the diagnosis and treatment of disease, and the practitioner who does not use clinical pathology well gives his patient unscientific and inferior medical service.

I hope you will let this pass for a definition of clinical pathologist, because when it comes to defining preclinical medicine, I find there is considerable confusion and that the literature of the subject is chiefly remarkable for what might be called "glittering generalities." In what has been written, there seems to be no distinction made between people who have no health complaints at all and people who have what Dr. Gould called "presymptoms." Obviously people who feel perfectly well are the only ones who can profit by periodic examinations. Those who have presymptoms like overweight, underweight, frequent colds, indigestion, rheumatic tendencies or tendency to shortness of breath, nervousness, and what have been hitherto generally considered negligible symptoms are not in need of routine or periodical examinations. They need treatment based on adequate diagnosis. I think it would improve matters to limit the periodic examinations to people who have no health complaints at all and to invent some other slogan such as "See your doctor early as no symptom is trivial enough to neglect," for people who have complaints and symptoms, no matter how slight they are, especially if there be any tendency for them to persist. With this distinction preclinical medicine would then be limited to those who have early or presymptoms.

Now the connotation involved in the concept "presymptom" is that borderline field between health and disease which good physicians are constantly struggling to narrow by making the diagnosis earlier and earlier. It is the most difficult of all fields of medical practice because the earliest evidence of malfunction is usually a symptom which has heretofore usually been regarded as negligible, and which we here call the presymptom. It is not so picturesque or impressive as the frank symptom of disease and therefore much more difficult to interpret and diagnose. Men who have devoted their lives to insurance work, however, are quite convinced that even the slightest of persistent symptoms or the faintest evidences of malfunction or the most benign of vicious habits affect longevity. In other words, preclinical medicine interprets and relieves, instead of neglecting, what have hitherto been regarded as negligible symptoms. It means, as Mr. Little says, "giving an individual continuous care instead of merely spasmodic attention on a breakdown."

Now, I admit that when I investigated the whole matter of health conservation some years ago with the view of constructing a plan which in operation would promise to prove most serviceable in conserving the health of people representing the general population, I spent five or six years getting what light I could on the matter by gathering statistics, with the help of expert statisticians and mathematicians in evaluating them. As a result it was impossible to come to any other conclusion than that by and large the practice of medicine throughout these United States more than anything else needed more and better facilities for giving people who were not considered actually sick the advantages offered by clinical pathology. Of course our viewpoint extended only over the field of preclinical medicine, but you will note that the result of our investigation led us to about the same con-

clusion regarding clinical pathology as Dr. MacEachern found in the clinical field. That conclusion was arrived at after studying thousands of examinations of apparently healthy people made by expert medical examiners and has since been substantiated by experience and by every bit of evidence that it has been possible to gather.

If you read any of the articles which have found their way into different journals, it makes no difference from what part of the country they have emanated, or whether they have been published by clinic or practitioner or some other kind of agency interested in health, and if you will examine the available statistics, you will see that in the preclinical field it almost always requires some kind of a laboratory examination to throw the necessary light on the great majority of cases which need medical help. Our study of an adequate human material has shown conclusively that the number of people who are healthy enough not to have to complain of symptoms or discomforts but who show physical signs on examinations are relatively rare as compared with the unexpectedly large number who are found to have significant health impairments by laboratory tests. Furthermore, the laboratory detects much more often than the physical examination changes which can be correlated with presymptoms, the evidences of slight malfunction or beginning disease. I was particularly glad that in our program for this meeting there were two papers on the subject of clinical pathology and dentistry, a very important subject in the preclinical field which is still in need of a great deal of constructive work.

The subject I have chosen is a very large one, but I am not going to attempt to cover it. I do, however, want to mention something that, from time to time, one sees in the medical journals. I am speaking of the activities of the life insurance companies and their interest in the promotion of longevity. Many of the articles show much confusion of thought, for instance in such a slogan as "no middleman is needed in practice." From reading them one gathers that there is a more or less widespread idea among physicians that life insurance companies have a kind of interest in the matter of preclinical medicine which is in its nature socialistic or tending to socialize medicine. I can speak with authority and know for a certainty, and believe that if you will think for a moment about it, you will not fail to understand that nothing can be further from the truth. In the first place it is inconceivable that insurance companies will ever for a moment think of practicing medicine or having anything to do with the treatment of sick people. The surpluses which they have the responsibility of accumulating for the benefit of policyholders offer too easy a target for unscrupulous or dissatisfied patients, and because there can be no practice of medicine without treatment, the intervention of insurance companies in the practice of medicine is an impossible absurdity. All that a life insurance company can ever hope to do in conserving the health of its policyholders is to try to do whatever it can to help policyholders get the best possible scientific medical treament instead of falling into the clutches of quacks, cults, and other fakers. Out of my own experience I can say with certainty that the active participation of life insurance companies in promoting longevity results not

only in discovering thousands of people in the early stages of disease at a time when they can be helped but also in finding large numbers of neglected people who have been discouraged or victimized by negligent and ignorant physicians, usually graduates of colleges happily no longer in existence. The cooperation of the life insurance companies in finding these people and trying to save them from the hands of fakery and ignorance would be regarded as a real accomplishment in the salvage of life if the truth were fully realized. From what I know about the attitude of life insurance companies, they constitute the staunchest bulwark that now exists against the introduction of anything socialistic into the practice of medicine, and every bit of evidence I have ever seen on the point conclusively shows this. Those of you who fear the menace of socialism in medicine should look in other directions and begin in our own ranks with the few traitorous physicians who compromise all of us by being greedy, incompetent, negligent or meddlesome. There are also other abuses; take the state laboratory, for example, which medical men are perpetuating into a real socialistic menace without realizing what they are doing. Certainly no time should be lost in making doctors understand that they are often unintentionally bringing about a drift toward socialistic tendencies. This drift, I feel convinced, does not happen as the result of any special design but simply as a consequence of incoordination between the different branches of the medical profession, especially those interested in public health activities.

Thus, only a few weeks ago the *Journal of the American Medical Association* carried a very good editorial. It seemed to me as if it had been written because some health officer complained of lack of cooperation between practitioners and health authorities. Of course the gist of it was that practitioners and the health authorities should cooperate. There is no other way about it; such cooperation is absolutely essential if we are to have a more complete and better understanding between the various branches of the medical profession. Once we have learned to coordinate our activities, a spontaneous cure of most of the difficulties which now occupy our minds will, I feel certain, be effected. Clinical pathology has suffered from lack of coordination and cooperation more than any other specialty of medicine and with the result that the public has not yet received its full measure of the advantages which adequate clinical pathology can yield. On the other hand, clinical pathology needs encouragement in order to advance scientifically more rapidly and to attract better and more men to it.

In closing, I desire not only to emphasize the value and necessity of educating medical practitioners regarding the advantages which clinical pathology affords them and their patients in the diagnosis and treatment of disease, but particularly to point out that clinical pathology also offers numerous possibilities for extending and improving the field of preclinical medicine, and that research along these lines can confidently be expected to produce important results in bettering the health of the nation.

ADDRESS*

By Rev. S. J. Moulinier

THIS was entirely unexpected on my part, but your esteemed President urged me to say a few words. I would like to have the opportunity of speaking to you tonight at your banquet because there are a few things that I have in mind that you might like to hear; not that there will be anything new to you, but they might have a little note of encouragement in them.

I want to congratulate your body of men on what you have been doing and what I am sure you are going to do in the future for scientific medicine. The practicing profession of the usual hospital is only slowly, as far as I am able to observe, becoming imbued with the final findings of the laboratory as signs in diagnosis and in treatment. All of you know this. I cannot tell you anything new. I would like to encourage you to develop the experience that you must have in a gathering of this kind and get it out into the field. I understand there are three or four hundred first-class pathologists. You all know how difficult it is for the hospitals to get men who are reliable, safe and sound clinical pathologists. You know, too, how difficult it is to get the staffs to have confidence in the pathologist. An organization like this, it seems to me, is bound to bring out a larger number, drawing them from the medical schools into this work. It is going to make the importance of the pathologist more clearly appreciated by the profession, and I think therefore in the next five, ten, fifteen years you will be what you should have been all the time, a specialist in medicine, consultants always in the hospital and relied upon and looked up to by the whole profession. I want to see the pathologist where he wants to be. There is hope of salvation for scientific medicine in the hospital. The College of Surgeons and our Association are working closely together trying to, what we call, standardize the hospital. It isn't the hospital that needs it so much; it is the medical profession. They need to realize more and more what scientific medicine is, and, as far as possible, be brought to practice it. Nearly every pathologist admits that ten years ago he was usually very discouraged about things; his laboratory wasn't being used as it should be, he couldn't get the men to appreciate it; he was seldom, if ever, taken into consultation; his work was looked upon as dispensable.

I hope you use your influence on the hospital phase of standardization and bring it about that the financial problem will not be so miserable as it has been in the past; that you will have better compensation, so that you can attract better men into your specialty; so that your number will grow in some way commensurate with the great number of hospitals. There are 7500 hospitals and 400 acceptable pathologists. What is being done to meet the situation? One man will have three or four hospitals. The fact that they are using men in so many places is an indication that they are coming to appreciate it more and more.

*Address to American Society of Clinical Pathologists, Sixth Annual Convention, Washington, D. C., May 14, 1927.

The College of Surgeons is giving its attention to the proper equipment and proper personnel of the laboratory. They started out using the word adequate laboratory service. They are coming to define what that means, in equipment and routine tests. But what I consider the best of future value I have mentioned; namely, that the staffs more and more are coming to look upon the pathologist as a real consultant on an equal standard with themselves. I am particularly sure that you all want that. It is the only standing for you to have. I want to thank you very much for inviting me to say these few words. Whenever I can I am going to further the interests of the clinical pathologist in the hospitals. You should get after them in the schools and the younger members of the profession so that there will be more and more coming into existence.

We are getting more and more interested in technicians. We want to follow out your rules and regulations and requirements for their training.

We can naturally help one another, and I know you will give me encouragement and help in what I am trying to do.

THE IMPORTANCE OF ORRIS ROOT AS AN ETIOLOGIC FACTOR IN HAY FEVER AND ASTHMA*

Based on the Study of One Thousand Cases of Hay Fever and Asthma

By Ray M. Balyeat, M.A., M.D., Oklahoma City, Okla.

SEASONAL hay fever has long commanded the intense interest of those working in the field of allergy. The interest is largely due to the peculiar and startling suddenness with which symptoms appear and the dramatic cessation of symptoms with the onset of frost. The relationship between pollen and seasonal hay fever is well established. There is another condition, however, which bears a train of symptoms similar to seasonal hay fever, but it is somewhat irregular in occurrence and has no relation to the month or season of the year. Recently careful attention has been given to the investigation of its cause. The terms rose cold, vasomotor rhinitis, hyperesthetic rhinitis, atopic coryza, perennial hay fever, and other names have been applied to the train of symptoms. Of these terms perennial hay fever is probably most descriptive. The causes of perennial hay fever are many, of which orris root is one of the most common. I wish to present some data relative to the importance of orris root as an etiologic factor in perennial hay fever, asthma, and other allergic diseases.

This work is based on the study of 1,000 cases of hay fever and asthma, of which 180 were hay fever and asthma cases in children, 82 were adult perennial hay fever cases, 365 adult asthma cases, and 373 were adult seasonal hay fever cases. They were all American born, private cases, ranging in age from four months to eighty-six years.

Of the hay fever and asthma cases under fourteen years of age 18, or 10 per cent, reacted to orris root. Of this number 3, or 1.5 per cent, were under

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five years of age, 7, or 3.9 per cent, were between five and ten years of age, and 8, or 4.4 per cent, were between ten and fourteen years of age.

From the above figures it seems that orris root sensitivity in allergic children is not uncommon. Two interesting case histories deserve brief discussion.

CASE 1.—D. W., male, age five years, has been a subject of perennial hay fever, eczema, and asthma for three years. The hay fever is much more severe in September and October. The mother has noticed that he would usually develop a congested nose and lacrimating eyes on attending the theatre, and the symptoms would occasionally be associated with a light attack of asthma.

Intradermal tests revealed the following:

Orris root	++++
Giant ragweed	+++
Short ragweed	++++
Western ragweed	++++

No other inhalant atopic substances were positive.

Treatment with an extract from orris root and one made up of the three ragweeds gave the boy complete freedom from symptoms.

CASE 2.—M. J. H., female, age three years, has suffered from eczema and asthma since three weeks of age and perennial hay fever during the past year.

Intradermal tests showed the following reactions:

Eggs	++++
Wheat	++++
Cat hair	+++
Orris root	++++

Removing the foods to which she was specifically sensitive from the diet and the use of unscented cosmetics in the home gave her entire relief from symptoms.

In the first case it appears that orris root was the sole cause of his perennial hay fever and asthma, but the ragweed produced typical seasonal hay fever. In the second case orris root was only a contributing factor. Later in life this child will naturally be brought in contact with much greater amounts of orris root, and in all probability will have to be desensitized against it if she hopes to be free at that time from her asthma and hay fever.

Of 82 perennial adult hay fever cases 39, or 47.5 per cent, reacted definitely to orris root. In 6, or 7.3 per cent, it was considered the sole factor. In 33, or 40.2 per cent, it appeared to be only a contributing factor. Any product that is either the sole cause or a contributing factor in 47.5 per cent of perennial hay fever deserves careful consideration. Two case histories relative to this condition will be briefly discussed.

CASE 3.—E. M. C., female, age thirty-two years, has suffered from lacrimating eyes in a moderate degree for three years. She complained of slight itching of the inner canthus of the eye and also had a mild congestion of the mucous membrane of the nose, at times with some itching of the nasal mucous membrane. She was advised by a competent eye specialist to be fitted with glasses, although he told her he found no special eye defect. She soon learned that wearing glasses did not change the symptoms of which she complained. Two other oculists were consulted, and each fitted her with a pair of glasses but without relief.

Intradermal testing showed a four-plus reaction to orris root but a reaction to no other atopic substances.

Changing her cosmetics to nonorris root ones relieved her of the lacrimating eyes and the congested nose.

CASE 4.—L. S., female, age thirty-five years, came to us several years ago with what appeared to be a chronic infection of the eyes. She complained of a congested and lacrimating nose, which was perennial in type. In July, however, her symptoms were very marked, typical of seasonal hay fever.

Protein tests showed a four-plus reaction to both western water hemp and orris root.

Changing her cosmetics to nonorris root ones, desensitizing her against orris root with an extract made from it, and treating her seasonal hay fever with an extract made of western water hemp has given her almost 100 per cent relief. Desensitization to orris root has been carried at from ten to fourteen day intervals over a period of four years. She has tried on several occasions to drop it, but symptoms of what seem to be a nasal cold will always appear. It is interesting to not that a dose of orris root will clear up the symptoms in from two to four hours when they appear.

Case 3 illustrates a type of mild sensitivity to orris root, requiring only the changing of her own cosmetics to nonscented ones and avoiding crowds. Case 4 is a severe one complicated with pollen sensitization.

Careful attention has been given to orris root as a possible factor in the etiology of asthma. Out of 365 asthmatic cases studied, orris root was the sole cause in only one but was considered one of the chief factors in 26, or 7.1 per cent, and a contributing factor in 48, or 10.4 per cent. In other words, it was one of the etiologic factors in 17.5 per cent of all our cases. During the investigation of an asthmatic patient careful testing should be done to rule out, or in, orris root as a possible factor. The following illustrative case histories are presented as evidence:

CASE 5.—B. B., female, age twenty-seven years, came to the clinic complaining of perennial hay fever all her life but worse during the past eight years. Associated with the hay fever symptoms were periodic attacks of asthma. The attacks of asthma had been diagnosed as pulmonary tuberculosis by a number of doctors. It is interesting to note that on putting her to bed in the hospital for rest, her chest findings would usually clear up in from three to six days and her nasal symptoms would be much better.

Both skin and intradermal tests to orris root were four-plus in reaction.

Elimination of orris root cosmetics gave but little relief as the routine of her life brought her in contact with others whose bodies and clothing were saturated with scented cosmetics. Desensitizing, however, allows her to attend the theatre and carry on her usual society functions with freedom from either hay fever or asthma.

CASE 6.—B. M., female, age thirty-seven years. Perennial hay fever began as a small child. During the last ten years she has had occasional attacks of asthma.

Protein tests were as follows:

Orris root	++++
Goose feathers	++
Chicken feathers	++++
Duck feathers	++

Changing her cosmetics and substituting kapok pillows for the feather ones helped but did not relieve her symptoms entirely. Desensitizing with an orris-root extract along with the above procedure gave complete relief.

Both patients mentioned entertain with social functions a great deal, naturally bringing them in contact with heavy concentrations of orris root. Both patients had had a number of nasal operations without relief. In Case 5 orris root was the sole cause of the asthma, while in Case 6 it was complicated

with a feather sensitization. In most cases of asthma or hay fever due to orris root, desensitization is required for relief, as removal of orris root from the patient or the patient from orris root is difficult.

Since seasonal hay fever is due to pollen, one might feel that orris root sensitivity would play no part in such a disease, but to find it a complicating factor is not uncommon. Of 373 cases studied, orris root reacted in 27, or 7.2 per cent. In all cases showing a marked sensitivity it was considered a complicating factor. It is not uncommon to find a patient, showing a four-plus skin reaction, who has no nasal or bronchial symptoms out of the hay fever season, but as soon as the nasal membranes are made hypersensitive by some pollen then the orris root becomes a definite factor. The following case will serve as an example.

CASE 7.—B. M. M., female, age forty-two years, has had ragweed hay fever for years but has never received much relief from the use of ragweed extract. She gave a four-plus reaction to giant and short ragweed, and also to orris root. She has no symptoms out of the ragweed season.

She was thoroughly desensitized with ragweed and advised to discontinue the use of scented cosmetics, which she did not do. She received about 40 per cent relief from her seasonal hay fever. She finally decided to use no scented cosmetics and found that her hay fever, although it was during the height of the ragweed season, disappeared.

This is a typical seasonal hay fever case whose orris root sensitivity gave no trouble except after the membranes were made hypersensitive by pollen. There are many such cases. For this reason orris root should always be thought of as a complicating factor in seasonal hay fever.

In the main, my findings are in accord with those of other men working in this field. Cooke[1] found 14.4 per cent of a series of 327 cases of asthma and hay fever sensitive to orris root. Spain[2] considers orris root an important factor in atopic coryza. Phillips[3] reported a series of 105 hay fever patients which was made up of both perennial and seasonal cases, in which orris root was the only factor found in 7, or 6.6 per cent, but it was considered a factor in 26 others, or 24.7 per cent. Rackeman[4] found 10.2 per cent of a series of 428 perennial hay fever cases sensitive to orris root. His findings are in marked contrast to ours. Of the series of 82 perennial hay fever cases which we studied, 40.2 per cent were sensitive to orris root. Rackeman, however, used the scratch method for testing, and the intradermal method was used by us, which in my judgment accounts for the difference.

In a study of the acquisition of specific hypersensitiveness, I have shown that a very large per cent of all hay fever and asthma patients are sensitive to more than one substance. It was also noted that the degree of contact with any given protein largely determines whether or not an individual with the inherent ability will become sensitive to that particular protein. The most common protein with which the mucous membrane of the nose and bronchial tubes of the average individual comes in contact is orris root; therefore a large number of people, who have inherited the ability to become sensitive, should develop a specific sensitivity to it. As a matter of fact they actually do.

In the year 1926 the women of the United States spent approximately $200,000,000 for scented cosmetics. Face powders are used very exten-

sively on the face and neck by the average woman and many of them dust themselves all over after bathing either with a scented tale or with some fragrant bath powder. There is a progressive tendency for women to wear less clothing and use more powder, and the scant, loose clothing permits the powder easily to fly in the air. A woman who uses an orris-containing powder soon saturates her clothing to the extent that not only herself but those about her are adequately exposed to orris root.

A reputable manufacturer of cosmetics has informed us that orris root is used rather extensively in the manufacture of a large per cent of face powders, face packs, astringent packs, cleansing powders and creams, scented tales, bath powders and salts, scented tooth powders and soaps, and that the orris oil is used extensively in preparing most synthetic perfumes and soaps.

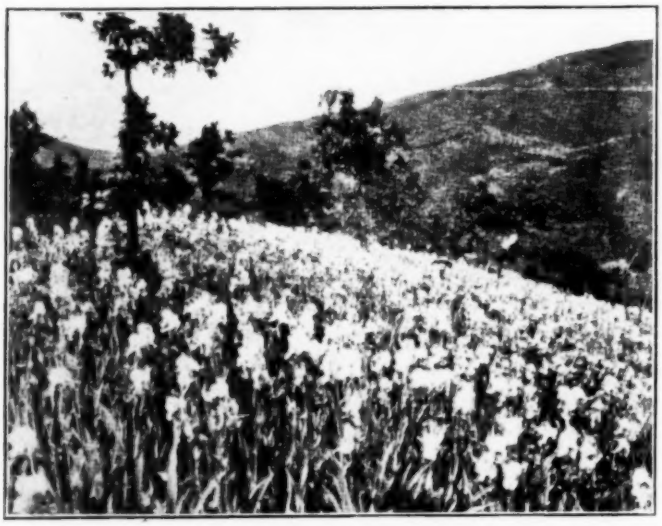

Fig. 1.—A field of iris plants near San Polo, Italy.

We have tested some of our orris root sensitive patients and find that their skins are sensitive to most of the scented cosmetics, which is excellent evidence that orris root is present in them.

Orris root is the rhizome of *Iris germanica, Iris pallida,* and *Iris florentina,* which is collected in the latter part of the summer, peeled, and dried in the sun. All three species are widely cultivated on the northern shores of the Mediterranean and other parts of Europe. The best quality roots are obtained from *Iris pallida* in Tuscany, and principally constitute the Florentine orris root of commerce. It is, of course, well known that Florentine rhizome is not synonymous with the root of *Iris florentina,* but includes all three varieties.

The district centering around the village of San Polo is renowned for the cultivation of the iris plant. Here practically every farmer grows iris as one of his crops, and from it he obtains the flowers, bulbs, and roots. The bulbs arise from the "eyes" that develop in the root, and vary in number according to whether the plant is a two-year or three-year plant. The flowers are

sold for ornamental purposes; the seeds do not reach maturity, and after cutting the flowers, the root undergoes more rapid development and becomes richer in its aromatic principle.

In San Polo the fields given over to iris have a southern aspect to insure the greatest amount of sunshine. The harvest of the rhizome is in June and August, at which time the season is dry, and the roots are well developed. The rhizomes are separated from the bulbs and are immersed in clean water to free them from adhering earthy matter. The bulbs are used for planting; the rhizomes are decorticated by the peasant girls and exposed to the sun to dry. The Florentine hills, in which San Polo is situated, produce by far the greater part of the total production of orris root. The rhizome is worked up for the various orris products in Milan, Grasse, and cities in Germany and England where cosmetics are manufactured.

Orris oil is obtained by distilling the various powdered rhizomes with superheated steam and condensing the vapors. A large per cent of the oil of commerce is produced in the neighborhood of Grasse, either from rhizomes grown there, or more frequently by distilling the roots imported from Tuscany. These two kinds are easily distinguishable, the French root yielding an oil with a much more delicate violet-like aroma. It is used very extensively in most synthetic perfumes and soaps. The function of powdered orris in the preparation of cosmetics is primarily that of a perfumed fixative, to make up and hold and slowly diffuse the essences of the scent employed; besides, it has an agreeable odor of its own and a flesh tint.

The methods of testing for orris root sensitivity are the same as those used in testing for sensitivity to pollen, animal epithelium, dusts, or foods. The skin tests should be used routinely, but in many cases a positive reaction will not be obtained by such a method. For this reason all cases should be checked by the intradermal method, which is a much more delicate one. Tests should be well controlled so that errors of interpretation will not be made. The solvent for the orris root should be used in making the control. It is my belief that every asthma and hay fever patient should be carefully tested with orris root, since one-sixth of all the asthma and hay fever patients we have examined are sensitive to it.

Hay fever symptoms due to orris root sensitivity vary greatly. Some orris root sensitive hay fever patients give histories which are somewhat similar to those of seasonal hay fever cases, but of course there is never any definite relation to the month or time of the year. Most orris root sensitive hay fever patients do not have the marked irritation of the eyes produced by pollen. Their symptoms are usually more marked when on the inside, especially when at church, the theatre, or in crowds at any time. Most seasonal hay fever patients can tell the exact time of the year and the exact year their symptoms developed, while it is not true, as a rule, in the case of the orris root patient, as the onset of symptoms seems to be gradual. Some patients appear to have a great deal more trouble during the summer, which is probably due to the increase in powders and cosmetics used by women during the summer months. The warm, dry air of the summer makes the orris root dust more buoyant, thereby allowing a greater concentration of it in the air. The

termination of hay fever symptoms, in those primarily sensitive to orris root, is not at the time of a killing frost as is true of those who suffer from a pollen sensitivity. In some cases the symptoms gradually grow less severe as winter comes on. In other cases, however, their symptoms are much more marked in the winter. The cold air, plus the irritation from the orris root, in many cases produces a greater amount of congestion of the nose than does occur in the summer. Symptoms, of course, depend to some extent on their occupation, their mode of life, etc. Many women live, during half of the year, in the climate of the steam-heated apartment, the average temperature of which is 75° F., and a relative humidity of 19. Such conditions are excellent for the dissemination of orris root, if it is used extensively in the home.

It is not uncommon to find orris root sensitive patients also sensitive to pollen, and at certain periods of the summer they suffer severely from typical pollen seasonal hay fever. Most patients who are extremely sensitive to orris root state that they are subject to frequent and sudden colds after being at the theatre, church, or any public gathering. In the winter many of these patients will complain of frequent colds, associated with itching of the nose, but on careful questioning one will usually find that the general aching which accompanies the average cold will not be present.

There was a time when we advised our orris root sensitive women to refrain from the use of cosmetics, but we soon learned that the vanity of women would not permit such advice to be carried out. It is now our custom to advise those who suffer from an orris root sensitivity to use no face powders, body powders, bath salts, sachets or soaps that are scented. A list of the inoffensive powders is given them. In cases that are only moderately sensitive to orris root, such elimination will cure their hay fever symptoms. However, about 75 per cent of the cases are so sensitive that the ordinary routine of life will bring them in contact with a sufficient quantity of orris root to produce symptoms, unless they are specifically desensitized against it. The process of desensitizing is similar to the one used in desensitizing patients who are sensitive to pollen. The treatment in our hands, and likewise those of others who are doing special work in asthma and hay fever, has proved very satisfactory. Orris root sensitive patients who complained of frequent colds in the winter, who received specific orris root therapy, usually reported freedom from winter colds.

Most doctors who treat hay fever and asthma mention orris root in their writings as a cause. The value placed upon it as a cause of hay fever and asthma by most of them is in accordance with our own ideas. However, until recently few have given it the space it deserves as a causative factor in an ailment which of late years has become distressingly common.

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THE ETIOLOGIC AND SPECIFIC RELATIONSHIP OF FOCI OF INFECTION TO CERTAIN ORGANIC LESIONS: A POSTMORTEM STUDY*†

By Alfred S. Giordano, M.D., M.Sc., South Bend, Indiana

IN A previous study[9] it was shown that bacteria recovered after death had a definite intravital significance. It became of interest then to determine whether organisms recovered from foci of infection and specific lesions at necropsy possessed a selective localizing power similar to that exhibited by certain bacteria recovered from similar locations during life.

The recognition of the part played by bacterial infections in the causation of chronic diseases has gained in importance with the multiplication of observations on the occurrence of such infections, in human beings, of ulcers of the gastrointestinal tract and analogous lesions of other organs associated with chronic foci of infection. Certain data for a proper appreciation of the relation between foci of infection and chronic infectious diseases can be more accurately obtained by a systematic bacteriologic study at necropsy than by clinical investigation during life. This is obvious when we consider that only at necropsy are certain regions accessible, such as the accessory nasal sinuses, the internal ear, and the central nervous system. These foci are available for direct examination and for cultural studies, and it is possible to rule out at once other regions as foci of infection; and still further, lesions whose etiology is sought can be accurately studied and the pathologic changes other than those in question may be established or eliminated.

Many have contributed to our knowledge of focal infection and the doctrine is now so firmly rooted as to become a tenet. Conspicuous among the contributions is the work of Rosenow[28] who has gone a step farther by enlarging our knowledge of the selective localizing power of certain bacteria. Focal infection as it occurs in man has been recently reproduced in animals by Rosenow and Meisser. These investigators have established foci of infection in dogs by devitalizing several teeth and filling the root canals with bacteria recovered from patients suffering from nephritis and nephrolithiasis, respectively; after a period of from two to four months, similar lesions were found in the dogs.

What determines the specificity of organisms is not clear. However, we do know that certain organisms, such as the bacilli of tuberculosis and of typhoid, when introduced into a suitable host produce specific reactions in certain organs. We also know that certain organisms have variable invasive

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powers for tissues. As Evans[7] points out, Treponema pallidum may invade the unimpaired mucous membrane of the lip, and the gonococcus the conjunctiva, while both of these structures are quite resistant to invasion by other organisms.

The technic used in culturing solid organs at necropsy has already been described in detail. I shall here describe the procedure used in obtaining the material from foci of infection and from lesions experimentally produced.

When infected teeth were removed, the gums and teeth were washed with 80 per cent alcohol, allowed to dry, and painted with tincture of iodine. The gums were then reflected and external alveolectomy performed with a mallet and chisel. The tooth was then lifted from its alveolus, and if any granulomas were present, they were curetted out and immediately placed in glucose-brain broth. The apex of each tooth removed was snipped off with pincers, or the entire tooth was wrapped in sterile gauze, crushed by a vise, and the root canal cultured. Sterile instruments were used throughout. If free pus was encountered in the socket of the tooth, it was taken up with a capillary pipette; some of this was cultured in glucose-brain broth and on blood-agar plates. The rest was suspended in normal sterile solution of sodium chloride for inoculation purposes. As a routine direct smears were made of the cultured material to rule out contamination.

When organs having relatively thin walls, such as the gastrointestinal tract, were being studied, the desired tissue, such as that of gastric ulcer, was excised with sterile scissors from the mucosa to the serosa and placed in the sterile salt solution and vigorously washed in several changes. If any portion of the tissue was contaminated, it was placed in a 1:1000 solution of mercuric chloride, and after six hours the excess was either washed off in several changes of sodium chloride solution or the sterile saline solution was allowed to run over the tissue for an hour on sterile wire gauze, various surfaces being exposed to the running solution. The tissue was placed in a sterile mortar and ground up with sterile sand, and cultures were then made of an emulsion of the macerated substance with normal sterile sodium chloride solution.

Animals were inoculated either with glucose-brain-broth cultures grown from twelve to eighteen hours, or with a suspension of the infected material in the sterile saline solution. Subcultures were rarely used for inoculation.

The reason for choosing young cultures that were not subjected to prolonged growth on artificial media is obvious when one considers the fundamental principles that govern the pathogenicity of bacteria, as laid down by Pasteur. He demonstrated that old cultures lose their pathogenicity and also that virulent organisms are attenuated when subjected to growth on artificial media and at minimal temperature. Rosenow has gone a step farther and shown that certain organisms, especially those in the pneumostreptococcus group, are very sensitive to differences in oxygen tension and to changes in the hydrogen-ion concentration. He has overcome these difficulties by the use of tall tubes of glucose-brain broth containing a buffer salt. I have adhered to these general fundamental principles closely in my technic under the supposition that changes in the pathogenicity of the organisms would also affect their specificity. Tissues were removed from the heart, liver, kidney,

and spleen, and from all evident lesions found in the animals. These were fixed in 10 per cent formalin, imbedded in paraffin, and the sections cut were stained by hematoxylin eosin and by the Gram-Weigert method. The material used for this study comprises cases of gastric ulcer, biliary lithiasis with cirrhosis, and ulcerative endocarditis.

GASTRIC AND RENAL LESIONS

Three cases of gastric ulcer were studied, one of which was complicated by renal lesions. The source of material in these three cases was from the teeth which were condemned by the attending physician and the dentist as foci of infection during the life of the patient.

CASE 1.—A man, aged sixty-eight, came to the Clinic complaining of involuntary and frequent urination. Initial urinary symptoms occurred five years before, and symptoms of duodenal ulcer had been present intermittently for twenty years. The anatomic diagnosis at necropsy included multiple gastric ulcer, duodenal ulcer, marked dental caries and sepsis and suppurative cystoureteropyelonephritis, with focal abscesses in both kidneys.

Around two of the six teeth removed aseptically periapical infection was found, and in two others infected granulomas. From the apexes of the teeth staphylococci and short-chain streptococci were cultured. These organisms were also found in the smears and cultures from the granulomatous material. Culture from the kidney yielded chiefly colon bacilli and staphylococci. Some rabbits were injected with glucose-brain-broth culture of an infected apex of a tooth, others with a normal sodium chloride suspension of the periapical pus; and still others with glucose-brain-broth culture from the patient's kidneys. The following protocols are typical of the results obtained. The lesions and their distribution in the other animals of the series are summarized in the Tabulation.

Rabbits 52 and 53, weighing 1 kg. each, were injected intravenously with 5 c.c. of a glucose-brain broth culture from an infected apex of a tooth containing almost a pure culture of green-producing streptococci and a few colonies of staphylococci.

Rabbit 52 was found dead at the end of thirty-six hours. Rabbit 53 was killed by chloroform forty-eight hours after injection. A careful, complete necropsy revealed areas of submucous hemorrhage and a clearly defined area of ulceration on the posterior surface of the greater curvature of the stomach extending through the muscular wall (Fig. 1). Viewed from the serous surface this lesion was grayish pink and sharply defined by a circle of punctate areas of hemorrhage (Fig. 2). Cultures from the blood, spleen, and bile were sterile while those from the kidney yielded only green-producing streptococci.

Rabbits 54 and 55 were injected intravenously with the pus suspended in normal sterile sodium chloride solution. This pus was obtained from the cavities after the removal of two teeth. Direct smears stained by Gram's method revealed pus cells, staphylococci and streptococci. Rabbits 58 and 59 were each injected intravenously with 5 c.c. of a twelve-hour glucose-brain-broth culture made from the pus.

Rabbits 54 and 55 died thirty hours later while Rabbits 58 and 59 were chloroformed forty-eight hours after injection. Necropsy revealed gastric lesions in all four animals while in Rabbits 55, 58, and 59 there were in addition many focal abscesses in the cortex and medulla of both kidneys (Fig. 3). Cultures from the blood and spleen were sterile, while those from the kidney and gastric ulcer yielded chiefly staphylococci, with a few colonies of green-producing streptococci. The two organisms were plated on blood agar and a pure culture of each was injected into five rabbits. It is interesting to note that in the animals injected with the subculture of staphylococcus only renal abscesses developed, and in the three animals injected with subcultures of streptococci focal hemorrhagic areas were found in the gastric mucosa. On the other hand, only in Rabbit 60, that was injected with a pure culture of streptococci not subjected to subculture obtained from the gastric ulcer of Rabbit 58, was a definite gastric ulcer found.

As previously stated, the culture from the patient's kidneys yielded chiefly colon bacilli and staphylococci. The colon bacilli were separated and the culture was injected

TABULATION

RESULTS OF ANIMAL EXPERIMENT

CASE NUMBER	ANIMAL NUMBER	ORGANISMS INJECTED	SOURCE	VEHICLE	ROUTE	ANIMAL PASSAGE	EFFECT ON ANIMAL	LESIONS IN					
								HEART	LUNGS	STOMACH	KIDNEY	JOINTS	SUBCUTANEOUS
1	R52	Streptococci	Tooth apex	G. B. B.*	I†	First	D‡	−	+	+	−	+	−
1	R53	Streptococci	Tooth apex	G. B. B.	I	First	Cl§	−	−	+	−	−	−
1	R54	Streptococci and staphylococci	Tooth apex	Saline	I	First	D	−	−	+	−	−	−
1	R55	Streptococci and staphylococci	Tooth pus	Saline	I	First	D	+	−	+	+	−	−
1	R56	Bacillus coli	Kidney	G. B. B.	I	First	D	−	−	−	−	−	−
1	R57	Bacillus coli	Kidney	G. B. B.	I	First	Cl	−	−	−	−	−	−
1	R58	Staphylococci and streptococci	Tooth pus	G. B. B.	I	First	Cl	+	−	+	+	−	−
1	R59	Staphylococci and streptococci	Tooth pus	G. B. B.	I	Second	Cl	+	−	+	+	−	−
1	R60	Streptococci	Stomach of R58	G. B. B.	I	Second	D	−	+	+	−	−	−
1	R61	Streptococci	Stomach of R54	G. B. B.	I	First	D	−	−	−	−	−	−
1	R62	Staphylococci	Kidney of R58	G. B. B.	I	Second	Cl	+	−	+	+	−	−
1	R63	Staphylococci	Kidney of R58	G. B. B.	I	First	D	−	+	−	−	−	−
1	R64	Staphylococci	Stomach of R54	G. B. B.	I	Second	Cl	+	−	−	+	−	−
1	R65	Staphylococci	Stomach of R54	G. B. B.	I	Second	Cl	−	−	−	+	−	−
1	R66	Streptococci	Stomach of R54	G. B. B.	I	Second	Cl	−	+	+	−	−	−
1	R67	Streptococci	Stomach of R54	G. B. B.	I	Second	Cl	−	−	−	−	−	−
2	R72	Streptococci	Tooth	G. B. B.	I	First	Cl	−	−	+	−	−	−
2	R73	Streptococci	Tooth	G. B. B.	I	First	D	−	−	−	−	−	−
2	R74	Streptococci	Stomach of R72	G. B. B.	I	Second	Cl	−	−	+	−	−	−
2	R75	Streptococci	Stomach of R72	G. B. B.	I	Second	D	−	−	−	−	−	−
3	R2255	Streptococci	Tooth	G. B. B.	I	First	Cl	−	−	+	−	−	−
3	R2256	Streptococci	Tooth	G. B. B.	I	First	Cl	−	−	+	−	−	−
7	R23	Hemolytic streptococci	Tooth	G. B. B.	I	First	Cl	+	−	−	−	+	−
7	R24	Hemolytic streptococci	Tooth	G. B. B.	I	First	Cl	+	−	−	−	+	−
7	R25	Hemolytic streptococci	Tooth	G. B. B.	I	First	Cl	+	−	−	−	+	−
7	R26	Hemolytic streptococci	Tooth	G. B. B.	I	First	Cl	+	−	−	−	+	−
7	R27	Hemolytic streptococci	Tooth	G. B. B.	I	First	Cl	+	−	−	−	+	−
7	R28	Hemolytic streptococci	Tooth	G. B. B.	I	Second	Cl	+	−	−	−	+	−
7	R29	Hemolytic streptococci	Tooth	G. B. B.	I	Second	Cl	+	−	−	−	+	−
8	R31	Streptococcus viridans	Tooth	G. B. B.	I	First	Cl	+	−	−	−	+	−
8	R32	Streptococcus viridans	Tooth	G. B. B.	I	First	Cl	+	−	−	−	+	−
8	R33	Streptococcus viridans	Tooth	G. B. B.	I	First	Cl	+	−	−	−	+	−
8	R34	Streptococcus viridans	Tooth	G. B. B.	I	First	Cl	+	−	−	−	+	−
8	R35	Streptococcus viridans	Tooth	G. B. B.	I	First	Cl	+	−	−	−	+	−
8	R36	Streptococcus viridans	Tooth	G. B. B.	I	First	Cl	+	−	−	−	+	−

*G. B. B. Glucose-brain-broth.
†I. Intravenous injection.
‡D. Animal died.
§Cl. Killed by chloroform.

intravenously into Rabbits 56 and 57. Both animals were found dead twenty-four hours later. Necropsy revealed no gross or microscopic lesions, but cultures from the blood, spleen, and kidney yielded colon bacilli.

In the kidneys of the patient, many focal and conglomerate abscesses were found. Many of the abscesses were relatively recent; others were in the

Fig. 1.—Rabbit 53. Multiple gastric hemorrhage.

Fig. 2.—Rabbit 75. Multiple gastric ulcer visible on peritoneal surface.

process of repair and still others were apparently entirely organized as indicated by the numerous scarred depressions (Fig. 4). The rabbit's kidneys in which focal abscesses were found correspond to those of the patient in distribution and acute reaction (Fig. 5).

IV. Intravenous injection.
AD. Animal died.
Kill. Killed by chloroform.

The gastric lesions of the patient were multiple and histologically the base of the ulcers was made up of scar tissue infiltrated with polymorphonuclears and lymphocytes. This infiltration extended deep into the muscularis. Sections from the lesions revealed Gram-positive cocci (Fig. 6). The ulcers produced in the rabbits were identical in distribution but could not be compared to those found in the patient because they were recent. However, it is interesting to note that two types of lesions were produced. In Rabbits 52 and 53, which were injected with cultures of streptococci, the lesions produced were apparently slight, consisting of areas of hemorrhage, desquamation of the mucosa (Fig. 1), and infiltration of the surrounding tissue with polymorphonuclear leucocytes and streptococci (Fig. 7). In Rabbits 54, 55,

Fig. 3.—Rabbit 59. Focal renal abscesses.

Fig. 4.—Necropsy on Patient 2-21. Focal renal abscesses.

58, and 59 which were injected with the suspended pus, containing both streptococci and staphylococci, the gastric lesions were different. Grossly the base and surrounding tissues of the ulcer were so markedly thickened as to attract attention to the presence of the lesion before the stomach was even opened (Fig. 2), while microscopically the lesion was found to be circumscribed or focal in character (Fig. 8) and to contain Gram-positive diplococci (Fig. 9).

CASE 2.—A man, aged sixty-nine, came with symptoms of gastric ulcer. Three weeks previously he had vomited blood. Perforating gastric ulcer was diagnosed. After appropriate preliminary medical treatment, the ulcer was excised by cautery and posterior gastroenterostomy was performed. At necropsy the chief pathologic lesions were bilateral broncho-

pneumonia, purulent bronchitis, and chronic diffuse nephritis. Several teeth pronounced diseased at antemortem dental examination were extracted at necropsy and granulomas were removed with strictly aseptic precautions.

Rabbits 72 and 73 were injected intravenously with 5 c.c. of glucose-brain-broth culture made from the granulomas. Rabbit 73 was found dead the following morning and was discarded on account of postmortem decomposition. Rabbit 72 was chloroformed forty-eight hours after inoculation, and necropsy revealed a grayish area, 8 by 4 mm., on the serosa of the greater curvature of the stomach, surrounded by petechial hemorrhages and edema. The corresponding mucosa of this area was found intact. The remaining organs were without evident lesion.

The tissue of the gastric lesion was excised and ground up with sterile sand. This material yielded a pure culture of indifferent streptococci.

Rabbits 74 and 75 were injected intravenously with the culture from the gastric ulcer of Rabbit 72. Both animals were chloroformed three days later, and necropsy revealed lesions

Fig. 5.—Rabbit 59. Focal renal abscesses (x40).

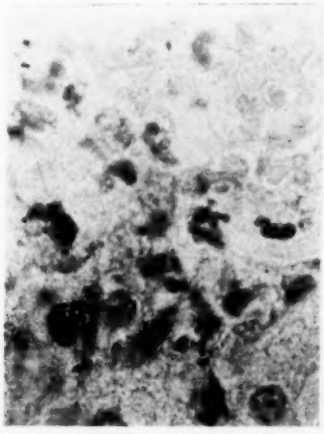

Fig. 6.—Necropsy on Patient 2-21. Gram-positive diplococci in base of gastric ulcer (x1000).

similar to those described in Rabbit 72 except that in Rabbit 75 the lesions were multiple (Fig. 2). Microscopically these lesions were characterized by diffuse polymorphonuclear infiltration of the muscular and serous coats while the mucosa was intact.

CASE 3.—A man, aged forty-eight, came to the Clinic with a history of symptoms characteristic of gastric ulcer. A large perforating gastric ulcer was excised. The patient died from bronchopneumonia. Culture of the blood, spleen, and peritoneum at necropsy yielded a pure growth of hemolytic streptococci.

The roentgen-ray and clinical dental reports indicated the extraction of the remaining upper teeth. Accordingly an infected tooth was extracted at necropsy under aseptic conditions, and cultures from the apex as well as the pyorrhea pockets yielded pure cultures of green-producing streptococci.

Rabbits 2255 and 2256, weighing 2 kg. each, were injected intravenously with 5 c.c. of glucose-brain-broth culture of pus from the alveolus of one of the teeth. Three days later the animals were chloroformed, and necropsy revealed punctate hemorrhages with an area of ulceration in the cardiac end of the stomach of both animals; the remaining organs presented no gross lesions.

Comment.—In the first passage through animals when the streptococcus alone was injected, only lesions in the stomach were produced. On second passage, when the organism was not subjected to subculture it retained its

Fig. 7.—Rabbit 53. Gastric lesions showing polymorphonuclear infiltration of submucosa and muscularis (x200) (see Fig. 1).

Fig. 8.—Rabbit 58. Focal necrosis in gastric wall (x100). (Gross lesion similar to Rabbit 75, Fig. 2).

specificity, while, when it was subcultured, the lesions were not as frequent and were less marked. When the streptococcus was injected with the staphylococcus, lesions of more marked and extensive character were produced,

while, when the staphylococcus was injected alone, it localized almost exclusively in the kidneys, and sometimes in the myocardium.

These cases illustrate the value of locating all the lesions which could be of focal origin in a given case. Had only the gastric lesions been known in Case 1 it would have been difficult to interpret the renal abscesses obtained

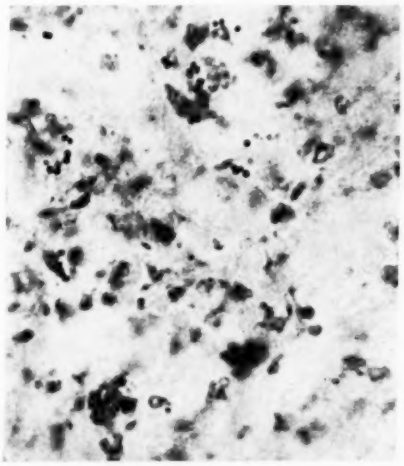

Fig. 9.—Rabbit 58. Gram-positive diplococci in base of gastric ulcer (x1000).

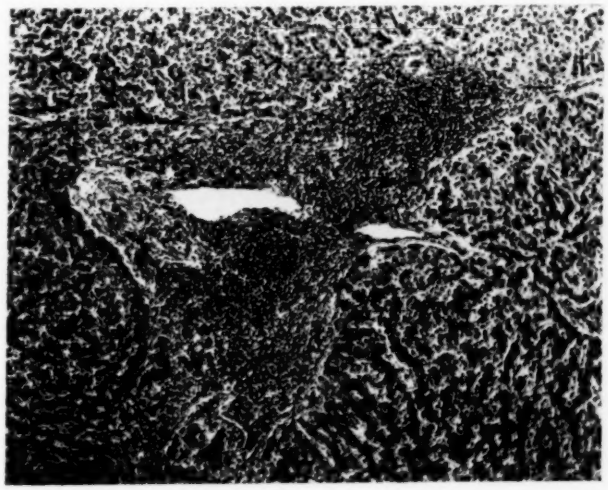

Fig. 10.—Necropsy on Patient 473 2-21. Liver showing marked periportal infiltration with obliteration of some bile ducts (x120).

in the animals. Thus, it is possible that in the antemortem study of lesions, focal in origin, which at times appear irrelevant, may actually have their counterpart in an unsuspected lesion. So far in the study of focal infection the streptococcus group has been given great prominence as an etiologic factor while the staphylococcus has usually been regarded as of relatively little consequence.

That the staphylococcus may enter the circulation from foci of infection in the nasopharynx was suggested by Billings and experimentally demonstrated by Rosenow and Ashby,[26] while Israel, Brewer, Jordan, McKenzie and Phemister have reported various lesions, such as osteomyelitis, renal abscesses and myositis, as being secondary to focal lesions in the skin. In reviewing records of 1,000 necropsies I have encountered two cases of diffuse myelitis and three of septicemia secondary to staphylococcic infection of the skin. Recently Meisser and Rosenow[18] showed experimentally in dogs that when teeth are infected with pathogenic staphylococci from the tonsils in a case of advanced nephritis, extensive lesions of the kidneys were produced. In view of the fact that staphylococci, when injected intravenously, produce lesions of the kidneys and other organs, and that when chronic foci of infection are established with these organisms, they actually enter the circulation, it is obvious that we should give more serious consideration to focal staphylococcic infections.

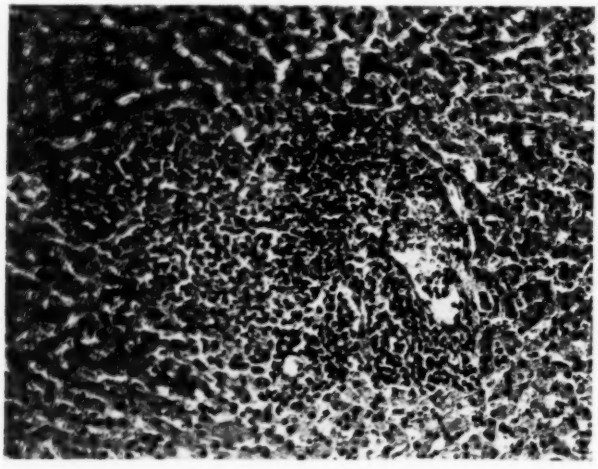

Fig. 11.—Dog 2. Periportal leucocytic infiltration involving bile ducts.

BILIARY LITHIASIS WITH CIRRHOSIS

Cirrhosis of the liver has, for many years, attracted the attention of the experimental pathologist, but up to the present time, the etiology of this not uncommon disease is as yet obscure. I shall here limit my brief discussion to the relation of bacteria to biliary lithiasis with cirrhosis.

Adami was the first to demonstrate that certain bacteria may cause cirrhosis. In the cirrhotic liver of cattle affected with enzoötic disease known as Pieton, he found a bacillus which he isolated in pure culture. However, when experimental animals were inoculated with it, they died before any changes occurred in the liver. Weaver, in 1889, isolated a Gram-negative bacillus from an incidental case of infectious cirrhosis of the liver in a guinea pig. This organism, when injected in large doses, killed the animals, but when injected in small doses, it produced a condition closely resembling cirrhosis

of the liver. Hektoen, in 1901, isolated a bacillus belonging to the pseudo-diphtheria group from a lesion of blastomycetic dermatitis. This bacillus injected subcutaneously produced cirrhotic changes in the liver. Unfortunately, as is often the case, both of these organisms soon lost their specificity, and complete studies could not be made. Nevertheless, in both cases

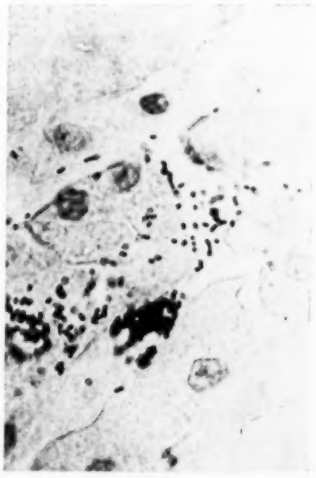

Fig. 12.—Dog 2. Gram-positive diplostreptococci in liver.

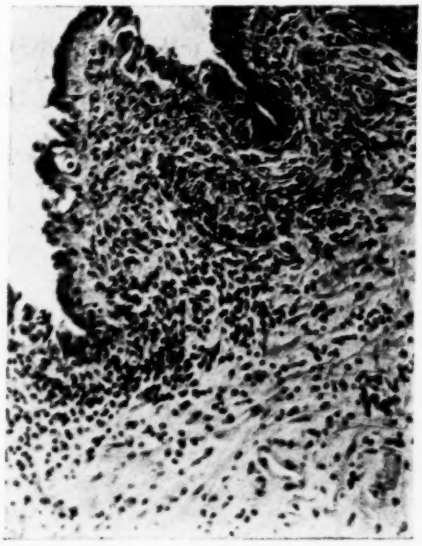

Fig. 13.—Rabbit A8. Gall bladder. Leucocytic infiltration involving entire wall (x120).

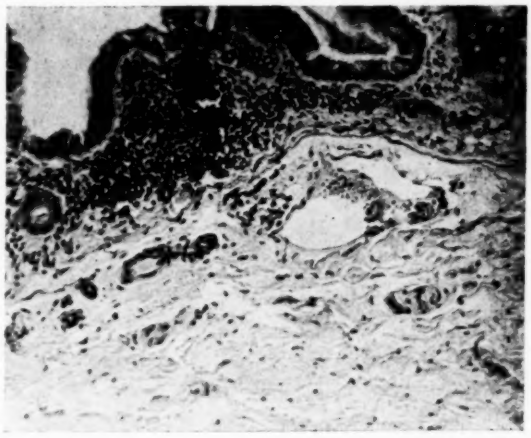

Fig. 14.—Dog 2. Gall bladder. Leucocytic infiltration (x120).

there was sufficient evidence to demonstrate that these organisms had a tendency to localize in the liver and produce a rather characteristic lesion. However, the specificity of some organisms is transient, owing perhaps to the unsuitable environment to which they are subjected during their growth on artificial media.

CASE 4.—A woman, aged fifty, came to the Clinic because she had had intermittent attacks of chills and fever with general abdominal pain for two years. For the six months previous to her admission these attacks were accompanied by slight jaundice and by clay-colored stools while her temperature varied from 94° to 104°. Examination revealed moderate icterus and a soft but slightly tender abdomen. The liver was 10 cm. below the costal margin in the midclavicular line. At operation the gall bladder was found shrunken around a mass of calculi which were also present in the cystic and common ducts. Although the calculi were removed from the common and major hepatic ducts jaundice persisted, and the patient gradually failed.

Necropsy, performed three hours after death, revealed no other abnormal findings except a large, firm, deeply bile-stained liver weighing 2,200 gm. The common bile duct was dilated and the hepatic ducts, from the porta hepatis to the periphery of the liver as far as they could be followed, were found to contain soft greenish concretions. Cultures from the heart, blood, and spleen were sterile. Cultures from the liver and biliary concretions yielded a pure culture of green-producing diplostreptococcus.

Fig. 15.—Dog 1. Soft concretions in gall bladder (x2).

Fig. 16.—Rabbit 27. Vegetations along margin of mitral valve.

Dog 1 was injected intravenously with 5 c.c. of a fourteen-hour culture obtained from the concretions found in the hepatic ducts. Rabbits A7, A8, A9 and A10 were injected intravenously with 3 c.c. each of a similar culture. Blood-agar plates of the injected culture yielded a pure growth of very fine green-producing colonies. Three days later the animals were killed by means of ether, and necropsy was performed.

The pleural cavities, lungs, and abdominal organs of Dog 1 were without gross abnormalities except for marked congestion of the liver; scattered throughout its surface were discrete hemorrhagic areas. On section the central veins stood out prominently. In the gall bladder was found 5 c.c. of greenish bile containing minute soft, greenish particles, the largest two being 4 by 8 by 3 mm., and 3 by 5 by 2 mm., respectively (Fig. 15). The gall bladder wall was markedly edematous. The common bile duct contained many minute sand-like bodies. Cultures were made from the liver, bile, spleen, and kidney, all of which yielded a green-producing diplostreptococcus. The blood culture was sterile.

Rabbit A7 revealed coccidiosis of the liver and was discarded. In Rabbit A8 all organs appeared to be without evident lesions, except the gall bladder which contained dirty greenish bile in which were suspended many fine greenish particles. Scattered throughout the mucous membrane there were discrete yellow pin-point areas of hemorrhage. Cultures from the liver, spleen, and kidney yielded similar green-producing streptococci. The blood and bile were sterile.

Rabbit A9 macroscopically resembled Rabbit A8 except that the gall bladder wall was thick and edematous. Cultures yielded similar results to those in Rabbit A8.

In the patient's liver the bile ducts were markedly infiltrated with polymorphonuclear leucocytes and lymphocytes. The hepatic cells near the periportal region showed various stages of disintegration. Some of the bile ducts were completely sclerosed while others were filled with epithelial débris (Fig. 10).

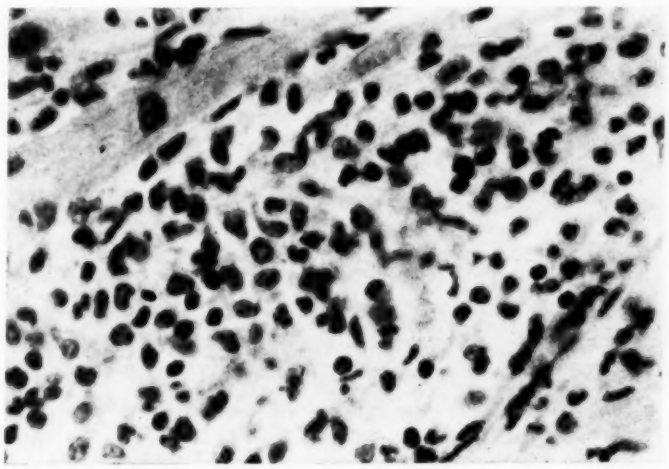

Fig. 17.—Necropsy on Patient 500A. Aschoff's body (x500).

Sections from the heart, kidney, and spleen of Dog 1 presented no noteworthy changes, while in the liver the epithelium of the bile ducts was desquamated, and in some the lumen was filled with polymorphonuclears and cellular débris. Scattered throughout the section were focal areas of necrosis which were most numerous around the periportal spaces (Fig. 11) and contained large foci of Gram-positive diplostreptococci (Fig. 12). The wall of the gall bladder was markedly edematous. The submucosa and muscularis were infiltrated with polymorphonuclear leucocytes, eosinophils, and lymphocytes (Figs. 13 and 14). Sections from Rabbits A8 and A9 were in general, similar to those of Dog 1, except that the leucocytic infiltration was made up chiefly of eosinophils and lymphocytes.

These experiments, though they prove conclusively that the organisms exhibited a marked tendency to produce lesions in the biliary tract, still permit the assumption that the lesions may have been preexistent in the animals.

However, another series of experiments was carried out with the organisms recovered from the liver of Dog 1 and the same technic, except that the inoculations were made after laparotomy had been performed under ether anesthesia. The gall bladder was explored after all of its bile had been evacuated with a syringe, and the walls were palpated for calculi. The physical properties of the bile were noted, and cultures were made from it. Then a small piece of liver was removed for microscopic examination.

Two dogs and two rabbits were studied. The gall bladders of all of these animals were free from calculi as far as could be determined by palpation at operation. The bile was clear, and no floating particles were present. It was sterile in all of the animals, and the microscopic sections of the liver removed for control were free from lesions. The animals were killed with ether on the third day after inoculation, and necropsy was performed immediately. They presented in general macroscopic and microscopic findings similar to those described in Dog 1, with only slight variations in extension of the lesions and absence of concretions in the gall bladder; the remaining organs were free from any gross or microscopic lesions.

Comment.—From these experiments it would seem, then, that the organism recovered from the liver and calculi in the bile ducts of the patient had a distinctive affinity for the tissues in the biliary tract, particularly the biliary ducts and gall bladder. The lesions produced in the animals closely simulated those found in the patient except that in the experiments the process was acute. The lesions in the liver were easy to control, but the interpretation of the concretions found in the gall bladder is difficult because even at laparotomy it is almost impossible to palpate small calculi; this is especially true when they are soft, as were those found in the animals. It would seem, then, that these organisms were etiologically responsible for lesions in the liver which may represent the early stage of biliary cirrhosis.

The results of these experiments were unexpected, and it was consequently too late to study the original focus, if any existed outside of the biliary system. Further, an attempt to repeat the experiments with the stock cultures was a complete failure; apparently the organisms had lost their specific property. Weaver and Hektoen had a similar experience.

ENDOCARDITIS

Perhaps the first one to demonstrate the presence of bacteria in vegetative endocarditis was Winge, in 1869. Rosenbach, in 1878, induced the disease in animals by passing a sound down the carotid artery and injuring the aortic valve. Later, Ribbert produced endocarditis by injecting staphylococci simultaneously with an emulsion of potato, while Orth and Wyssokowitsch, and Fulci obtained similar results by using pulverized carbon simultaneously with staphylococci or streptococci. The following year, Bonome, and Josserand and Roux produced ulcerative endocarditis by injecting staphylococci alone intravenously. Dreschfeld, using streptococci, obtained similar results which were later confirmed by Poynton and Paine, Davis, Jackson, and Rosenow.[27] Rosenow attributed to the organisms a selective affinity for the endocardium. The observations of Weichselbaum, Wright and Stokes, and Henrici

have definitely proved that no one organism is specific for the lesion, although some may produce endocarditis more frequently than others, as pointed out by Poynton and Paine and by Rosenow. The possibility that endocarditis and rheumatic fever were secondary to focal infection was first definitely proved by Poynton and Paine, and corroborated by Rosenow's extensive research on foci of infection, including teeth, tonsils, and pharynx. Later, Hartzell and Henrici, and Moody demonstrated that alveolar infection around the teeth contains organisms of variable degrees of virulence which, when injected intravenously into animals, produce definite lesions that closely resemble those that existed in the patient.

The following two cases, studied experimentally, are of interest not only because of the relation of focal infection to endocarditis, but also because they illustrate that the same condition can be produced in man and animals by different strains of organisms, and they may also throw some light on the etiology of rheumatic endocarditis and allied conditions.

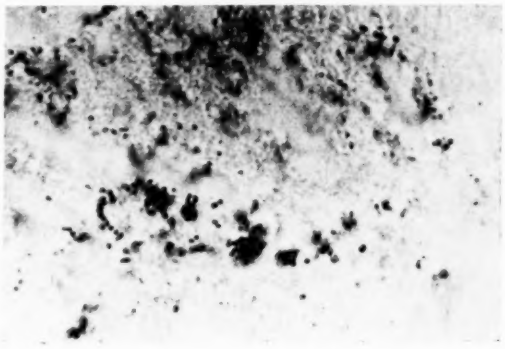

Fig. 18.—Necropsy on Patient 500A. Gram-positive streptococci in margin of vegetation (x1000).

CASE 7.—A woman, aged about thirty, gave the history of having had recurring attacks of cardiac failure associated with fever. One month before death she had chills and fever and shortness of breath. The teeth were found by roentgen ray examination to be diseased, having ten periapical abscesses. The heart was enlarged, and a presystolic murmur was present. The condition gradually became worse and two days before death petechial cutaneous hemorrhages and right hemiplegia appeared. At no time during the whole illness did the patient complain of pain in the joints. Necropsy, performed five hours after death, revealed extensive chronic and acute vegetative endocarditis and infarction of the left cerebral hemisphere, spleen, and both kidneys. Cultures from the blood, brain, spleen, and kidney yielded a pure culture of hemolytic streptococcus.

Six of ten infected teeth were removed and cultures made from the infected granulomas of four; all of these yielded hemolytic streptococci which corresponded culturally and morphologically with the organisms recovered from the blood and other organs.

Four rabbits were injected intravenously with organisms recovered from the infected granulomas. In three of them multiple purulent arthritis, vegetative endocarditis, and focal myocarditis were found, while in the fourth animal there were only a few hemorrhagic areas along the line of closure of the mitral valve. The organism was recovered and passed through another series of three rabbits, with similar results in all (Tabulation).

Rabbit 27, weighing 850 gm., was injected intravenously with 3 c.c. of twelve-hour glucose-brain-broth culture from an infected granuloma of a tooth. Three days after inoculation the right knee joint was swollen and painful on slight pressure. The animal did not

touch the ground with the right limb. The next day it became very ill, and was chloroformed. Scattered throughout the endocardium of the left ventricle were numerous grayish areas varying from 1 to 2 mm. in diameter, surrounded by red zones. In the base of the mitral valve and along the line of closure there were raised reddish areas varying from 1 to 3 mm. in diameter (Fig. 16). The right knee joint was swollen and contained about 3 c.c. of cloudy fluid. The joints of the right and left fore limbs also contained cloudy fluid. In the smears made from the joint fluid were found pus cells and Gram positive streptococci. Cultures from the blood, spleen, and joint fluid all yielded hemolytic streptococci in pure culture.

CASE 8.—This patient had attacks of rheumatic fever with involvement of joints and definite mitral stenosis with auricular fibrillation. Necropsy revealed healed and fresh vegetations along the line of closure of the mitral valve. Two definite infected granulomas were obtained from three teeth. These yielded green producing streptococci in pure culture. Rabbits were accordingly injected. The lesions in the remaining animals injected are noted in the tabulation.

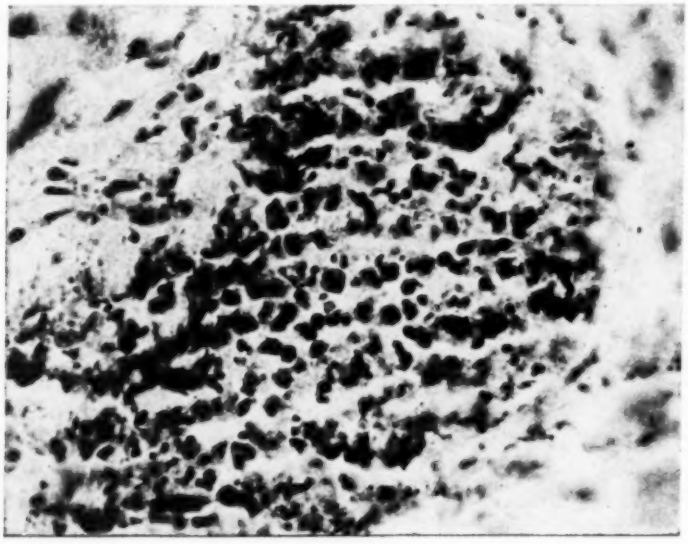

Fig. 19.—Rabbit R27. Perivascular infiltration of subendocardial nodules (x200).

Rabbit 34, weighing 1.2 kg., was injected intravenously with a twelve hour glucose-brain-broth culture containing green-producing streptococci.

Two days later the animal was chloroformed. Necropsy revealed discrete areas of hemorrhage in the endocardium of the mitral valve and left ventricle. In the line of closure of the mitral valve there was a definite area of ulceration 2 mm. in diameter, and scattered through the wall of the left ventricle were discrete grayish areas.

Microscopically the punctate grayish areas described in the endocardium of the left ventricle in Cases 7 and 8 were found to extend for a short distance into the myocardium in the intermuscular septa. The areas were focal and made up of groups of large endothelial cells, lymphocytes, and an occasional polymorphonuclear leucocyte arranged close to a vessel (Fig. 17). No bacteria could be demonstrated in these areas. The vegetations were made up of necrotic tissue, fibrin, leucocytes and large numbers of streptococci (Fig. 18). The lesions found in the myocardium of the rabbits corresponded identically with those of the patient, in being focal in type and

perivascular in distribution, but the predominating cells were polymorphonuclear leucocytes and lymphocytes (Fig. 19). The Gram stain revealed large numbers of diplostreptococci in the center of the lesions (Fig. 20).

Comment.—The myocardial lesions associated with endocarditis have given rise to considerable discussion in regard to their resemblance to the Aschoff bodies which, according to some authors, are believed to be specific for rheumatic endocarditis. Aschoff, in 1904, first described a focal myocardial lesion associated with rheumatic endocarditis and called it "submiliary endocarditis of rheumatic fever." The lesion which he described was multiple and occurred in the intermuscular septa closely associated with a blood vessel; it was composed of a group of large oval or spindle-shaped cells which occasionally were multinuclear and arranged in the form of a rosette or a fan. These cells were surrounded by lymphocytes and a few polymorphonuclear leucocytes. Geipel, a year later, described similar nodules in cases of rheumatic fever although he did not admit their specificity since he also found them in a case of nephritis associated with interstitial myocarditis. Thal-

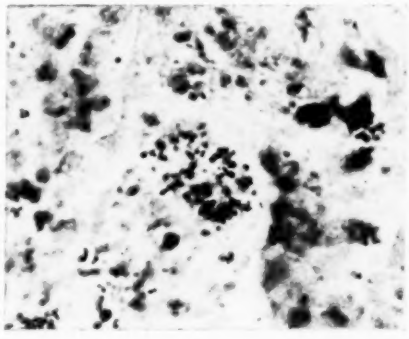

Fig. 20.—Rabbit R27. Gram-positive diplostreptococci in same area as Fig. 19. (x1000).

heimer and Rothschild believe that the presence of Aschoff bodies in the myocardium is strong presumptive evidence of rheumatic fever, even though no history is available.

The experimental evidence submitted by various investigators is not entirely in harmony. Bracht and Wachter maintain that they have reproduced these bodies in rabbits inoculated with streptococci isolated from patients with rheumatic fever. The lesions described were focal in character and contained lymphocytes. Jackson made a similar study of myocarditis produced by injecting a strain of hemolytic streptococci isolated during a milk epidemic. The organism, when injected intravenously, had a marked tendency to produce purulent arthritis and focal myocarditis. The age of the lesions studied in this series varied from two days to about two months, and it is of interest to note that the early lesions contained numerous polymorphonuclear leucocytes and bacteria as well, while in the later lesions lymphocytes and oval cells predominated with an occasional giant cell; in the old lesions only fibrous scars remained. Thalheimer and Rothschild believe that the Aschoff bodies are found only in cases of rheumatic fever and chorea, and are absent

in cases of subacute endocarditis, due to Streptococcus viridans. They admit that these organisms, when injected intravenously into rabbits, produce focal myocarditis, but they maintain that the cells in these lesions are different in structure and staining reaction with pyronin methyl-green. They believe also that the focal myocardial lesions are of toxic origin since they were unable to demonstrate bacteria. Hartzell and Henrici, in a recent study on the specificity of streptococci, found that a certain percentage of various strains of streptococci of low virulence ingested produce focal myocardial nodules which, although not always of similar character, could not be histologically differentiated from Aschoff bodies; they concluded that the lesion is not specific since it was produced by several strains of streptococci. However, they agree with Thalheimer that the myocardial lesions are of toxic character, since they also were unable to demonstrate bacteria in them.

The two cases cited here represent two different clinical and pathologic pictures: one of vegetative endocarditis (Case 7) and the other, of what is clinically classified as rheumatic fever associated with endocarditis (Case 8). In both cases the manifestations were probably secondary to periapical dental infection; in the myocardium in both cases focal perivascular lesions were found which histologically resemble the submiliary endocarditis of rheumatic fever described by Aschoff. The organisms recovered in each case were culturally different, and when injected intravenously into animals, tended to localize in the myocardium and endocardium; they produced focal, perivascular miliary necrotic areas containing streptococci. The arthritis produced by the hemolytic streptococci was distinctly more pyogenic in character than that produced by the green-producing streptococci.

It would appear, then, from the review of the literature and my own experiments, that in all probability, the Aschoff bodies are not characteristic of rheumatic fever. Perhaps the reason for the disagreement in the comparison of the lesions is that the time factor has not been emphasized. Possibly the earliest lesion is focal perivascular necrosis in which polymorphonuclear leucocytes predominate, due to the chemotactic substances liberated. During this stage, bacteria can be demonstrated. As the lesion becomes chronic, the bacteria are destroyed; the lymphocytes and endothelial cells predominate and eventually only a scar is left.

CONCLUSIONS

1. There is definite evidence that organisms recovered at postmortem examination from foci of infection and specific lesions may exhibit a selective localizing power.

2. Such selectivity is comparable in all its phases, including animal inoculation, with that which has already been proved by other studies. This fact constitutes an additional argument in favor of careful routine and exhaustive bacteriologic examinations of all cases studied at postmortem

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OCCURRENCE OF LIPOIDS IN URINE AND THEIR DIAGNOSTIC IMPORTANCE*

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UNDER the term lipoids we mean fatty substances, which are composed chemically of cholesterol compounds, mainly cholesterol esters, and optically show anisotropic properties. Kaiserling and Orgler were the first to discover double refracting fat substances morphologically in human tissues, which subsequent investigators recognized as cholesterol esters. At the present time several microchemical methods are at our disposal for their histologic demonstration and differentiation. The above-mentioned authors were the first to observe the presence of lipoids in the urine removed during the autopsy of a nephritic patient.

On this occasion I am considering only that group of fatty substances, which are double refracting, and I am omitting any discussion regarding their chemical nature as well as their microscopic differentiation. For my investigations I employed the Reichert's polariscopic attachments.

The examination of a urinary sediment with this apparatus may reveal the following findings:

1. A minute, double refracting granule, either isolated or found in groups.

2. A cast, consisting mainly of anisotropic material, which is termed lipoid cast.

3. Small epithelial cells, apparently desquamated cellular elements of the tubular apparatus of the kidney, which contain double refracting substances in their protoplasm.

4. Larger, foamy cellular elements including anisotropic fat substances, derived from blastomatous growths in the genitourinary tract.

The more cholesterol esters are present in a fat globule, the more pronounced will be the anisotropic phenomenon, and the sharper will be the polarizing cross figure.

The first question to be answered is: Do the normal renal parenchyma and the normal urine contain double refracting substances?

The exact and extensive histologic investigations of several research workers proved that lipoids occur in neither the tubular apparatus nor in interstitial tissue of the normal kidneys. Personally I have examined the urine with polariscopic attachments in 380 cases of both male and female individuals in various decades of life, who did not present clinically any renal symptoms and whose urine when chemically analyzed was normal with the exception of an occasional faint trace of albumin; in no case did I succeed in de-

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teeting double refracting substances in the sediment. Von Noorden, Munk, and Geneck, also emphasize the fact that in normal urine no cholesterol esters can be found.

One must admit theoretically, however, that under normal conditions the blood cholesterols may be excreted through the kidney (alimentary transitory lipoidemia), but in such minute quantities as to defy detection completely; consequently this occurrence would not carry with it any practical significance.

Even during certain pathologic conditions, which are accompanied by a hypercholesterolemia we are not able to observe lipoids in urine as long as the kidney is not affected. In connection with this fact the experiments of Weltmann and Biach, and Geneck are of particular importance, since during the course of an artificial hypercholesterolemia these authors succeeded in finding lipoids in urine, only after the kidney had been previously damaged with uranium nitrate. The occurrence and ready detection of lipoids in urine, therefore, represents a pathologic condition, which is called lipoiduria or cholesteroluria.

Now, the question arises under what circumstances can lipoids be found in urine?

In the first place and mainly in the so-called genuine lipoid nephrosis, a peculiar nosologic entity, accurately described by Munk, characterized by the development of a general edema, marked albuminuria, hypercholesterolemia and lipoiduria, by the absence of high blood pressure and hematuria. The urine sediment is loaded with double refracting fat substances, lipoid casts, and with lipoid-bearing cells. It is most likely a metabolic disease, a primary disturbance of cholesterol metabolism, its real nature being still undetermined.

Certain cases of lipoid nephrosis may develop acutely during the secondary stage of syphilis, as it was originally pointed out by Munk and Fahr. The urine sediment at this time is rich in double refracting substances and lipoid casts. These findings in combination with a nephrotic symptom complex and a positive Wassermann blood reaction characterize an acutely developed primary lipoid nephrosis. In the blood we may also find an increase of lipoids probably indicating a disturbance in lipoid metabolism.

Occasionally, however, during the salvarsan-mercury treatment toxic lesions of the kidney may suddenly occur, with a subsequent appearance of lipoids in the urine. These instances must be sharply separated from the cases of primary lipoid nephrosis. The differential diagnosis must be based upon an exact clinical analysis and observation (functional kidney test).

Lipoids in urine may also occur, as it was first emphasized by Munk, in chronic and subacute cases of glomerulonephritis, in fact approximately eight weeks after the acute onset, but never during the acute stage. This indicates that nephrotic changes start to accompany the nephritic process. In certain cases of genuine and atherosclerotic shrinkage of the kidneys lipoids may appear in urine, their occurrence being uncommon, even irregular. Munk states further that in cases of acutely developing parenchymatous degenerative changes of the kidneys urine does not contain double refracting substances.

From these observations and contentions we may advance the following rule: The presence of lipoids in urine in a case of a pure nephrotic symptom complex proves a primary genuine lipoid nephrosis. In nephritic cases, however, their appearance in urine signify secondary degenerative or infiltrative deposits of lipoids in the tissues of the kidney, a secondary lipoid nephrosis.

Dr. F. D. Murphy, who, upon my suggestion, has made considerable clinical and pathologic studies of cholesterol in various forms of nephritis, points out, that those cases of chronic diffuse glomerulonephritis which were associated with edema, were characterized by the presence of lipoids in the tubular epithelium of the diseased kidneys. But on the contrary, those cases which were not accompanied by edema did not disclose any deposits of anisotropic substances in the cellular elements of the renal tubules. Murphy believes that an association exists between glomerulonephritis with edema and an elevation of the blood cholesterols, deposits of lipoid material in the tubular epithelium, and the presence of double refracting substances in the urine. His investigations are in complete accord with the findings of Munk. In a series of fifty cases of heart disease with edema Murphy was unable to find any abnormal amounts of cholesterol in the blood and no lipoids in the urine, indicating herewith the possibility of differentiation between a cardiac and a renal dropsy.

Several authors (Finger and Kollert, Geneck, Knack) questioned the statements of Munk regarding the absence of lipoids in urine in cases of acute glomerulonephritis since they apparently succeeded in finding double refracting substances in the urinary sediment during a very early stage. It is, however, questionable whether these investigators were absolutely familiar with the exact time of the commencement of the acute nephritic process.

A further pathologic condition of the kidney, in which the lipoids may play an important diagnostic rôle, is amyloidosis. In amyloid degeneration of the kidney, in amyloid nephrosis, we meet with cases which show a rich deposit of double refracting substances in the interstitial tissue of the kidney and very negligible amounts in the renal tubular apparatus. On the other hand we know of instances, where mainly the cellular elements of the tubules contain lipoids, occasionally to such an extent that one can easily recognize and follow the structure of the kidney parenchyma in the dark-field during the micropolariscopic examination. The latter cases are clinically accompanied by a nephrotic symptom complex and by a cholesterolemia.

In connection with this statement I want to mention the observation of Fahr, who found large deposits of cholesterol esters in the kidneys in each of his nineteen cases of amyloid nephrosis with edema, while the double refracting substances were absent in cases without edema.

The amyloid nephrosis can be assumed with the greatest probability if, during the course of certain wasting diseases (such as chronic purulent affections of bones or joints, severe ulcerous types of tuberculosis, persistent bronchiectatic suppurations, etc.), the nephrotic symptom complex appears. We can, therefore, clinically differentiate between a simple amyloid degeneration of the kidneys and an amyloid nephrosis.

Certain types of malignant neoplasms of the urogenital tract may occasionally lead to lipoiduria, as the so-called hypernephroma or Grawitz tumor

of the kidney, especially in those instances, when the tumor mass perforates into the pelvis of this organ. The tumor tissue is rich in fatty substances, which partly exhibit anisotropic character. Their detection in urine, in combination with clinical manifestations, may be of some help to the urologist while endeavoring a diagnosis.

A peculiar kind of primary carcinoma of the prostate, known as lipoid carcinoma, may produce lipoiduria. This tumor variety was first described by Schlagenhaufer, a Viennese pathologist. I had the opportunity to examine a similar case three years ago at St. Joseph's Hospital, Milwaukee, Wis. If the tumor penetrates into the lumen of the bladder, the urinary sediment may contain tumor cells which are loaded with lipoids and also isolated double refracting substances in the form of minute granules.

In adult male individuals, particularly in advanced age, the prostate contains lipoids under normal conditions, which are found in the epithelial elements as well as in the interstitial tissue. The same is true in many instances of the so-called hypertrophic, adenomatous prostate. Dr. W. M. Kearns examined under my direction a large series of such prostates and confirmed these findings. In my own micropolariscopic examinations I did not succeed in finding lipoids in urine in cases of so-called prostatic hypertrophy.

Finger and Kollert have apparently seen lipoids in the urinary sediment in a case of pyelonephritis and are inclined to question their clinical diagnostic importance. Far more significant is the fact that Geneck investigated thirty-three cases of cystitis, pyelitis, and cystopyelitis and found only negative results.

Lately during postmortem examinations of two cases, which showed a pronounced hypercholesterolemia, I found yellow, xanthoma-like nodules, about the size of a pea, in the chorioid plexus of both lateral ventricles, consisting mainly of cholesterol crystals, formations which I designate as cholesteroma. Sorry to say I neglected to examine the lipoid content of the cerebrospinal fluid. These findings may lead us to believe that these tumor-like cholesterol deposits in the chorioid plexi are probably concomitant with an increased presence of lipoids in this fluid, influenced by a disturbance in cholesterol metabolism.

While summarizing these brief statements, permit me to emphasize that lipoiduria, the occurrence of double refracting fat substances in urine, occurs in the following pathologic conditions:

1. Lipoid nephrosis of unknown origin (genuine lipoid nephrosis).
2. Lipoid nephrosis of luetic etiology (secondary stage of syphilis).
3. Subacute and chronic glomerulonephritis (combination forms of nephritis and nephrosis).
4. Amyloid nephrosis with edema.
5. Grawitz tumor (so-called hypernephroma) of the kidney.
6. Lipoid carcinoma of the prostate.

I have presented only a brief outline of that which one could consider on this very important subject. In reviewing the textbooks of clinical laboratory methods we are unable to find any information about this matter. My

aim was to arouse the interest of clinical pathologists in this question and to stimulate further observations and studies. I want further to emphasize the practical importance of the examination of the urinary sediments with polarizing microscope for clinical diagnostic purposes, and to recommend it as a routine procedure for every pathologic urine.

DISCUSSION

Dr. Wm. G. Exton.—This matter is a very important one. We have extracted a number of albuminous urines with alcohol and ether, and I have been surprised at the number of urines from which fat could be extracted by simply shaking them with ether or alcohol and ether.

Dr. Mortimer Herzberg.—I wish to know if Dr. Miloslavich has any observations on the evidence of fat in a tuberculous kidney or in conditions of pernicious anemia.

Dr. Wm. G. Exton.—It has been confusing to me that lipoids might be found in the urine which do not show in the sediment. In a number of instances we have not been able to see them in the sediment but have been able to extract them with alcohol and ether. The presence of lipoids in urine is certainly worthy of investigation, and I hope Dr. Miloslavich will pursue his studies and further enlighten us as to methods and significance.

Dr. Miloslavich (closing).—The technic is a very simple one; the polariscopic attachments can be applied to any microscope. The urinary sediment is then used in the ordinary way. One point of interest would be to investigate whether or not the temperature of the urine influences the demonstration of the presence of lipoids. In cases of pernicious anemia and advanced tuberculosis, one may find presence of isotropic fat substances.

INCIDENCE OF VARIOUS SPECIES OF BACTERIA IN SPINAL FLUIDS FROM CASES OF MENINGITIS*†

By Ruth Gilbert, M.D., and Marion B. Coleman, B.S., Albany, N. Y.

A COMPILATION of the results of the routine examination of spinal fluids over a period of seven years has been made in order to determine the incidence of various species of bacteria in spinal fluid from cases of meningitis in New York State outside of New York City. As the district served is largely rural, it is of interest to compare our figures with those furnished by Neal[1] and Dunn,[2] who have reported specimens from cases in urban districts.

Since January 1, 1920, in two hundred and eighty-four instances, organisms of pathogenic significance have been isolated. The figures in Table I represent the number of patients from whom specimens were submitted, two or more specimens frequently having been received from the same person.

Table I

Species of Bacteria Isolated from Cases of Meningitis

Reported by	Tubercle bacilli	Meningococci	B. influenzae	Pneumococci	Streptococci	Other bacteria	Total cases
Neal, New York City	662	625	51	86	83	16	1523
Dunn, Boston	60	60	4	12	6		142
Gilbert and Coleman, Albany	167	18	23	33	35	8	284

The number of tuberculous spinal fluids (167) is relatively very high. The age incidence, however, corresponds to that determined by others, eighty-nine of the patients being under ten years of age. A study of the specimens from this group of children under ten years of age has been made to determine whether the disease was of bovine or human origin. Rabbit inoculations with 0.01-mg. amounts of culture in thirty-one instances have indicated ten strains to be of bovine origin and twenty-one of human origin. The study of the other strains has not been completed.

The relative percentage of specimens from which meningococci were isolated is very low. Of the eighteen strains reported, there were three corresponding serologically to Gordon Type I, three to Type II, one which was agglutinated equally well in Types-II and -III sera, and one which was agglutinated in all three sera. Seven others were morphologically and cultur-

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†From the Division of Laboratories and Research, New York State Department of Health, Albany.

ally typical of meningococci and were agglutinated definitely in the polyvalent meningococcus serum, but were not agglutinated in Gordon Types-I, -II, or -III sera. These would be classed as "X" atypical, corresponding to the strain reported by Kirkbride and Hutton[3] in 1926. The three remaining strains were classified as meningococci because they had morphologic and cultural characteristics of meningococci and appeared to be the incitants of meningitis. They were not agglutinated in polyvalent meningococcus serum. The fact that meningococci isolated from sporadic cases of meningitis are serologically less uniform than those found in an epidemic, has been frequently reported by other workers. The relative infrequency of the isolation of meningococci may have been due to the length of time, usually one or two days, which our specimens are in transit. However, specimens containing large numbers of polymorphonuclear leucocytes in which no bacteria can be demonstrated are rarely received, and in only four instances were morphologically typical meningococci found in the spinal-fluid sediment when they were not found on cultural examination.

Pfeiffer's bacillus was found in twenty-three instances and, as noted by others, the majority of cases (17) occurred in children two years of age or less. There was no indication in the histories accompanying these specimens that the meningitis followed any type of respiratory disorder except that two were from children who were recovering from whooping cough. As is generally known, the mortality of influenzal meningitis is high. In a review of two hundred and twenty cases, Rivers[4] reports only seventeen recoveries. It has been our experience that this type of infection is definitely indicated by the presence of large numbers of very pleomorphic Gram-negative bacilli, together with large numbers of polymorphonuclear leucocytes in the spinal-fluid sediment, and a tentative diagnosis may be made on these findings. The work of Wollstein[5] and Rivers and Kohn[6] indicates that the strains of B. influenzae found in meningitis are closely related serologically. Serum treatment in experimental influenzal meningitis in monkeys was used by Wollstein[7] with satisfactory results. Torrey[8] reports the recovery of two cases in children after serum treatment; Neal,[9] one after vaccine and serum treatment; and Jousset and Girard,[10] one after vaccine treatment alone. Therefore, it would seem that if satisfactory serum or vaccine treatment could be perfected, the ability to make an early diagnosis would permit its effective use.

Pneumococci and streptococci were found in thirty-three and thirty-five instances respectively. In only three of the pneumococcus infections was a history of pneumonia given. Sixteen of the pneumococcus strains isolated were Type IV; thirteen, Type III; two, atypical Type II; and two, Type I. Among the specimens containing hemolytic streptococci were two from meningitis following head injuries, and three, following mastoid infections. Seven of the streptococcus strains were of the variety producing methemoglobin. These are included because this organism was present in pure culture and was found in the original film preparations of the sediment.

In a few specimens organisms not commonly associated with meningitis were found; i.e., B. typhosus in one instance (the history did not indicate

whether this was a typical case of meningitis or one of typhoid showing meningeal irritation), Micrococcus catarrhalis in two instances, and B. pyocyaneous from a patient with meningitis following a fractured skull. In four other instances, unidentified organisms were found, their presence possibly being due to contamination.

In conclusion, it is important to note the relatively low incidence of meningococci in this series, which is lower in fact than that of B. influenzae. As the mortality in cases of meningitis due to B. influenzae is very high and there is some evidence that vaccine or serum treatment might be of value, a further study of such agents may be indicated.

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LABORATORY METHODS

A NEW AND RAPID METHOD OF EXAMINING TISSUE MICROSCOPICALLY FOR MALIGNANCY*

By Benjamin T. Terry, M.D., Nashville, Tenn.

INTRODUCTION

NEW Method.—Not all tumors can be diagnosed in the gross. Even our best surgeons need from time to time a quick microscopic examination for malignancy. Up to the present the most rapid method has been that of frozen sections. In the hands of those who have acquired the necessary skill and experience, frozen sections are dependable. But against their use many objections have been raised. It is feared that the freezing may produce misleading artefacts. The method is expensive, and the services of an expert technician are required. Even under good conditions the sections are sometimes torn, wrinkled, or distorted. As a consequence of these and other objections, many experienced pathologists do not use frozen sections at all. There is a real need for some easier method which will permit a quick microscopic diagnosis of tissue and yet will avoid most of the objections raised against frozen sections. In this paper such a method is described.

HOW METHOD WAS EVOLVED

The new method is the outcome of experimentation stretching over a period of nearly eight years and is the direct result of a visit paid the Mayo Clinic in the summer of 1919. At that time Dr. William Carpenter MacCarty showed me the most beautiful frozen sections of fresh unfixed tissue I had ever seen. The technic employed had been introduced in 1905 by Dr. L. B. Wilson,[1] and the stain was Unna's polychrome methylene blue. Before the war, according to Dr. Wilson, the best samples had been obtained from Germany, but at the time of my visit it was almost impossible in this country to purchase good German stain. To ripen it at room temperature I was told required from six months to a year or more, and many batches spoiled in the ripening.

As I wished to use the stain, I tried to learn how to ripen it more rapidly. This I succeeded in doing by incubating the alkalinized stain at 37.5° C. for six days, and this method was adopted at the Mayo Clinic.[2] Further experiments showed that at a higher temperature the time could be reduced to a few minutes. The stain can now be ripened by boiling for sixty seconds.[3]

Alkaline polychrome methylene blue is excellent for frozen sections of fresh unfixed tissue, but is not so satisfactory for frozen sections of fixed tis-

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sue. As most of the tissues I examined were fixed in formalin, experiments were carried out to produce a stain that would be equally satisfactory on fixed tissue. These experiments were also successful.[4] Subsequently it was found that by altering the concentration of the stain for fixed tissue, it too could be ripened by boiling for sixty seconds.[5]

The satisfactory results obtained with these two stains on frozen sections of fresh and fixed tissue suggested their use on tissues studied in the gross. As a consequence thick slabs of tissue were cut, stained, and examined by reflected light. The results were surprisingly good. With low powers of the microscope small areas of malignancy[6] could often be picked out. In many instances, however, the low powers merely raised a suspicion of malignancy. To be certain of the diagnosis, higher powers were essential.

Improvements in technic permitted magnification up to 100 or 150. To make this possible, special lamps[3] were constructed and so attached to the microscope that a beam of strong light was directed obliquely downward upon the upper surface of the stained sections. For assistance given me at this stage of my work I wish to acknowledge with thanks my great indebtedness to the optical firms of E. Leitz in Wetzlar, Germany, and Bausch and Lomb in Rochester, New York. These firms designed or adapted lamps that I could easily and successfully use.

For a time, however, all attempts to get satisfactory magnification above 150 diameters were in vain. An accidental observation eventually led to success and to the discovery of what I am inclined to believe is a new principle in microscopy. One day I was struck by the fact that occasionally some thick stained sections examined by transmitted light were ever so much better than others. On attempting to find the explanation, all of the good sections were carefully examined, and it was found that these had one thing in common. All showed one side well stained, but the other side was stained poorly or not at all. This irregularity in staining was accidental, but to determine its significance, I now stained other sections, taking care to have the stain on one side only. The result was most gratifying. Thick sections stained on one side only and examined by transmitted light often showed nuclei and other histologic details as satisfactorily as if they were very thin sections. The explanation seemed simple. If the sections are stained on one side only and very superficially, light can easily be transmitted through the other unstained side, thus causing the stained cells to stand out sharply on an illuminated background. On the other hand, if the section is stained on both sides, light transmitted through the first surface is altered in quality, and it may be so completely absorbed that too little gets through to illuminate the upper surface sufficiently. The correctness of this explanation has apparently been amply demonstrated by the tests to which it has been subjected.

New Principle.—Instead of having to cut tissue very thin to get histologic detail, by this new principle all that is necessary is to stain relatively thin sections superficially on one side only with a good precise stain and then examine the moist tissue, stained side uppermost, under a thin cover, using transmitted light. With fixed tissue astonishingly beautiful microscopic pictures were obtained.[7]

Unfixed Tissue a Problem.—When, however, the same technic was applied to fresh tissue, the results were not satisfactory. Unfixed tissue is usually so soft that it is difficult to section. Moreover, under the microscope the stained surfaces often showed numerous unstained areas. The rapid microscopic diagnosis of unfixed tissue presented a real problem.

Two possible solutions suggested themselves: (1) Some new method of rapid yet perfect fixation might be worked out, or (2) the technic for fixed tissue could in some way be altered to make it suitable for fresh unfixed tissue.

Quick Fixation.—The simpler and easier solution seemed to lie in quick fixation. It is well known that heating fresh tissue for a short time in formalin often enables one to secure satisfactory results if frozen sections are cut and stained with hematoxylin and eosin. But polychrome methylene blue is a very sensitive stain. For it to act well, the tissue apparently must either be unfixed or well fixed. Its use on specimens heated in formalin seemed to indicate that tissue that was *quickly* fixed was *imperfectly* fixed. With none of my many attempts at rapid fixation was I well satisfied.

Characteristics of Unfixed Tissue.—With a view to adapting the fixed tissue technic to fresh tissue, the characteristics of the latter were studied. Fresh unfixed tissue differs from fixed tissue in at least five ways: (1) It is usually softer. To cut it properly the razor should be as sharp as possible. (2) The cells of unfixed tissue are much easier displaced or dislodged. If after staining some are displaced, the surface under the microscope will show areas which appear to be unstained. To avoid losing cells from the surface, the greatest care must be exercised in cutting, staining, washing, and mounting. (3) Unfixed tissue frequently decolorizes the stain. If the stained tissue is to be seen at its best, it must be examined at once. (4) Unfixed tissue is more translucent than fixed tissue. This is a great advantage, for it permits transillumination of thicker sections. (5) Some fresh tissues are very red due to the presence in them of many red blood cells. Red tissues are difficult to examine satisfactorily, for the blue rays in the examining light are absorbed.

New Technic.—With the characteristics of unfixed tissue in mind the technic has been modified until it is now applicable to many tissues, although not to all. The details of this new technic will be given after the advantages and disadvantages of the new method have been pointed out.

ADVANTAGES

Quickly Learned.—The technic is simple, easy, and quickly learned. Anyone with good eyesight, intelligence, and a little mechanical dexterity can acquire the necessary technic in a few minutes.

Extremely Rapid.—In favorable and easy cases the microscopic diagnosis can frequently be given in less than sixty seconds.

Uses No CO_2.—As no CO_2 is required, the usefulness of the method is widened. It can be employed where no CO_2 can be secured, where the supply is exhausted, where the services of an expert frozen-section technician are not available, or where the freezing apparatus is out of order.

Inexpensive.—The new method is inexpensive. If one already has a microscope the other things necessary can be secured for a few dollars, and the cost of operation and upkeep is almost nothing.

Transportable.—The equipment is simple, compact, light, and readily transportable. The method seems ideal for use in the doctor's office, or in an emergency in the patient's home.

Noiseless.—As the method is noiseless it can be used in the operating room. For this purpose it seems to be the best yet devised.

Timesaver.—The new method saves the time of the surgeon, the pathologist, the technician, and the patient.

1. The sooner in doubtful cases the surgeon receives his tissue report, the sooner he can complete his operation. If the surgeon is promptly assured that a given piece of tissue is or is not malignant, his operation is planned and carried out on the basis of definite information. If he can feel sure that malignancy is not present, he can often substitute a simple and less radical operation for the one he would have done if he had not received a very prompt tissue report. The new method is apparently the fastest yet proposed.

2. As a rule the pathologist cuts enough blocks of tissue to be reasonably sure that in one or more he will find the changes necessary for his diagnosis. The more difficult the case seems, the larger the number of blocks taken. Often eight or ten are excised when one would suffice if the pathologist could know at once what it shows. By the new method a microscopic diagnosis can be secured on each block as it is taken. The pathologist then preserves only those which are of value. As his diagnosis is reached sooner, he saves time and tissue.

3. By having to section or embed for permanent record a smaller number of blocks, much of the technician's time is saved. There is also a big saving in reagents, slides, and covers.

4. Patients wish to get well quickly and be out of the hospital as soon as possible. If in a number of cases the new method permits the surgeon to perform less radical operations, the time and other interests of the patient are conserved.

Few Artefacts.—The method is comparatively free from artefacts. The tissue is examined perfectly fresh. There is no freezing, fixing, heating, or dehydrating. Probably many of the cells are still alive. In any event smooth muscle in the stained sections often shows lively contraction. By this new method cells are studied as nearly as possible as they are in the living body. This is a great advantage.

No Special Lamp.—With the new technic no special lamp is necessary. Under favorable conditions strong daylight suffices, but a good, efficient artificial light is better.

High as well as low powers of the microscope may be employed.[7] If the technic is good, oil immersion examination of the tissue is possible and satisfactory.

Dependable.—The method gives dependable microscopic pictures. When the technic is carefully carried out and the diagnoses are made by a skilled and experienced pathologist who, until he is thoroughly familiar with the new method, controls all of his diagnoses by the older methods, there is little occasion to fear that malignancy will be missed, or that it will be diagnosed where it is not present.

Main Value.—While it is probable that the new method will prove to be most useful in studying malignancy, it is by no means limited to this. Acute and chronic inflammatory changes are often clearly seen. The method should be of great value in selecting tissues to be examined by other methods, in studying tissues from autopsies, in checking doubtful diagnoses, and in identifying unlabelled tissues.

Advantages of New Stain.—By substituting for the alkaline stain a neutralized polychrome methylene blue, the method here described works very satisfactorily both on fresh and on formalin fixed tissues.[7] This is a great advantage, for fixed tissues are often easier to cut and diagnose than some fresh unfixed tissues.

In Studying and Teaching.—The new razor section method should prove of value in the study and teaching of cytology, histology, and pathology. It should also be useful on fixed tissue. Students of pathology often wish to see sections of normal tissues. By keeping on hand portions of normal organs fixed in formaline, it is easy to cut, stain, and mount sections of them whenever they are called for.

Tissue Not Wasted.—The preliminary cutting of razor sections does not prevent the use subsequently of other methods of diagnosing. Even thin razor sections are usually thick enough to be cut on the freezing microtome or embedded and cut in paraffin or celloidin. Moreover, the staining of sections with polychrome methylene blue does not interfere with the subsequent staining of these with other stains, for the polychrome is extracted completely when the tissues are run through alcohol.

DISADVANTAGES

The new razor section method for fresh unfixed tissue has a number of disadvantages.

Not Permanent.—As the tissue is not fixed, the sections are not permanent. In this respect they resemble frozen sections of fresh unfixed tissue. To secure permanent sections some other method must be employed.

Quick Fading.—The sections fade very quickly. They must, therefore, be examined immediately after staining. The sooner they are seen, the more beautiful they appear. Faded sections can, however, be repeatedly restained.

Limited Applicability.—While the method is suitable for the examination of the majority of unfixed tumors, it is not now easily applicable to all. At present it is also unsatisfactory for examining some unfixed normal tissues. This limitation in applicability is the method's greatest disadvantage. With further improvements in technic, it is to be hoped that this drawback will be reduced or overcome entirely.

Difficult Tissues.—When examined in the fresh unfixed state, tumors and other tissues may be difficult for five reasons. They may be hard to *cut, stain, mount, examine,* or *diagnose.*

1. They are hard to *cut* if they contain much calcium or bone, if they are extremely soft, or small, or friable, or if they are composed of numerous small polypoid masses.

2. They are hard to *stain* if necrotic or if they are covered with mucus or colloid.

3. They are hard to *mount* if very small or extremely narrow or ragged or if the tissue is composed largely of living smooth muscle. This latter by its contraction, often throws smooth sections into many folds.

4. They are difficult to *examine* if light cannot be readily transmitted through them. Opaque tissues or those which are darkly pigmented are difficult to transilluminate. Moreover tissues that are red are also difficult. Very porous tissues and sections which have rough surfaces due to improper sectioning act in a similar way, for they allow the stain to sink quickly into the depths. When in these cases transmitted light is used, it has to pass through a stained layer before it reaches the upper surface and this stain in the depths acts like a color screen, absorbing most of the light.

5. They may be hard to *diagnose* due to inherent difficulties. Borderline tumors often necessitate prolonged study. As razor sections stained by polychrome methylene blue fade quickly, it does not seem probable that this new method will be especially useful here, although in its favor is the fact that it permits the rapid study of many sections from different parts of the tumor.

Not Projectable.—Razor sections cannot yet be satisfactorily projected. They are too thick and they fade too quickly. On account of the rapidity with which they are decolorized by strong light, it is even difficult to get good microphotographs.

Reasons for Fading.—For the rapid fading of the stained razor sections there are apparently three reasons: (1) As the sections are relatively thick and the staining is at first very superficial, the unstained cells in the neighborhood of the stained ones soon begin to rob them of stain; (2) many living tissues quickly decolorize the stain; (3) the light used to examine the sections also decolorizes them.

Restaining.—Sections that have been decolorized more or less completely can be restained. The restaining can be repeated several times, but eventually becomes unsatisfactory due to the tissues in the depths becoming stained.

This list of disadvantages is long, but it should not discourage, for most tumors and many other tissues are easily examined. If there is no hurry in making the diagnosis, the difficult tissues should be fixed in formalin and examined again by the razor method.

Interesting Parallelism.—It is worthy of note that the cases which have been difficult by the new method have, as a rule, been difficult also for frozen sections. Sometimes one method shows a little advantage, sometimes the other.

TECHNIC

The technic described in this paper is the result of many experiments. If the directions here given are followed carefully, satisfactory sections should be obtained.

Equipment.—This is simple and inexpensive. In addition to the microscope the following things should be on hand: A good sharp razor, a hone, and a strop. A small piece of cork measuring about 10 by 10 by 0.5 cm. or larger, some pins, and a good pair of pointed forceps capable of picking up a hair. One or two tumblers full of distilled water or of clear cold tap water, a bottle of good neutral polychrome methylene blue, a medicine dropper, a good strong artificial light, some clean cover glasses and microscopic slides, and one or more slides of the same size, made of glass or wood, but

Fig. 1.—A simple but efficient arrangement of the inexpensive equipment.

differing from the ordinary slides in having a round or oval hole 2 cm. or a little larger cut entirely through the center. These special slides are convenient but not necessary.

The razor should be kept as sharp as possible. Whenever it becomes the least bit dull, it must be honed and stropped. An experienced barber can quickly teach you how to sharpen it effectively. The importance of keeping the razor very *sharp* can scarcely be overestimated. The kind of razor is less important. I have used a number of different ones and all have been satisfactory *if sharp enough*. Even safety razor blades and microtome knives have been tried. The Durham Duplex razor blade is convenient and is safe to use if one edge is first covered over with a little adhesive plaster to lessen the danger of the operator cutting himself. The Sextoblade is also excellent. Best of all, however, in my experience is the old-fashioned razor which is still used by most barbers.

A thin piece of cork is used as a cutting board. Into this cork, pins are stuck to immobilize the tissue. A table mat of compressed cork is excellent and can be purchased for ten cents from some Woolworth Store.

The Stain.—Unless the stain is good, the results will not be satisfactory. Alkaline polychrome methylene blue does not keep long. If there is the least doubt about its quality, it should be made up fresh according to directions already published.[3] These have been tested many times, and if carefully followed, they give good stains. It is advisable to use c. p. anhydrous carbonate and medicinal methylene blue. Other brands may be good, but sometimes the substitution of another has led to failure.

Directions for an improved stain are found in the footnote.*

Many lamps have been tested. One which is very inexpensive but quite satisfactory is a 60 watt Mazda lamp with frosted white bulb. Occasionally when the sections are thick or are overstained, a stronger lamp is better.

The slides with holes in them can be easily made in a few minutes out of cigar box wood. The illustrations accompanying this paper show what they look like and how they are used. If sufficient care is exercised in cutting the tissue to make the surfaces plane parallel, ordinary slides and covers can be used.

It is very important to keep the razor blade clean and smooth. It should be wiped immediately after using. If the razor is rusted or has clotted blood or bits of dried tissue clinging to it, the surface of the tissue will be scratched and the microscopic picture will be less good than it should be, for there will be displacement of the surface cells.

Technical Steps.—These are ten in number.

1. Selection. If malignancy is to be looked for, the pathologist as soon as the tissue reaches him, should select by careful inspection, palpation, and section, the area or areas he wishes to examine microscopically.

2. Excision. Cut out the area selected, bearing in mind the subsequent steps of immobilization and slicing. If possible the block should include an advancing edge of the tumor. A convenient size is 1 by 1 by 0.5 cm., although

*Neutralized Polychrome Methylene Blue.

This stain keeps well and acts rapidly, precisely, and differentially on both perfectly fresh unfixed tissue and on formalin fixed tissue. There are five steps in making it. (1) The preparation of stock solutions A, B, and C. (2) The titration of Solution A against Solution C. (3) The alkalinization of B by means of A. (4) The polychroming of the alkalinized B. (5) The neutralization by means of C of alkali already added to B.

The first five steps in detail are as follows:

1. The three stock solutions in neutral distilled water are:

A.	12% K_2CO_3, C.P. anhydrous,	100 C.C.
B.	1% methylene blue, medicinal,	1000 C.C.
C.	10% acetic acid, by volume,	100 C.C.

2. Titration. Determine how much of Solution A exactly neutralizes 1 c.c. of boiling standard Solution C, using phenolphthalein as an indicator. Mark this quantity on Bottle A.

3. Alkalinization. Into a 100 c.c. graduate, place that quantity of A, which is equivalent to 1 c.c. of C, add enough of B to make 100 c.c., and mix thoroughly.

4. Polychroming. Of the alkalinized methylene blue 25 c.c. are placed in each of four one ounce bottles. These are stood unstoppered in cold water which is brought to a boil in about 10 minutes. Note the time, and remove the bottles one by one 15, 20, 25, and 30 minutes later. Let them cool slowly. The water after reaching the boiling point should be kept boiling while the bottles are in it.

5. Neutralization. To each 25 c.c. of the polychrome stain add 0.25 c.c. of Solution C.

Filtration is usually unnecessary and should not be carried out immediately. The four bottles are polychromed differently to permit each worker to make and choose stains which best suit his own taste, light, and work.

Two firms in New York City, the National Aniline and Chemical Company, 40 Rector Street, and Elmer and Amend, Third Avenue at 18th Street, have begun to make the stain here described, and each has agreed to submit samples for my approval before marketing it.

smaller or larger blocks may be successfully cut. A small block properly excised is much better than a larger one not so carefully selected.

3. Immobilization. If tissue is to be sliced thin and smoothly, *it should not move while it is being cut.* The block is immobilized by carefully pinning its broadest and flattest surface to the cork plate, using two or more pins, the number depending on the size, shape, and consistency of the tissue. The illustrations will show how the pins are used. The tissue may be further supported by forceps. If the technician is right handed, it is convenient to pin the tissue 2 to 3 cm. above and to the left of the lower right corner of the cork. If the block consists mainly of soft tissue but has a dense capsule or

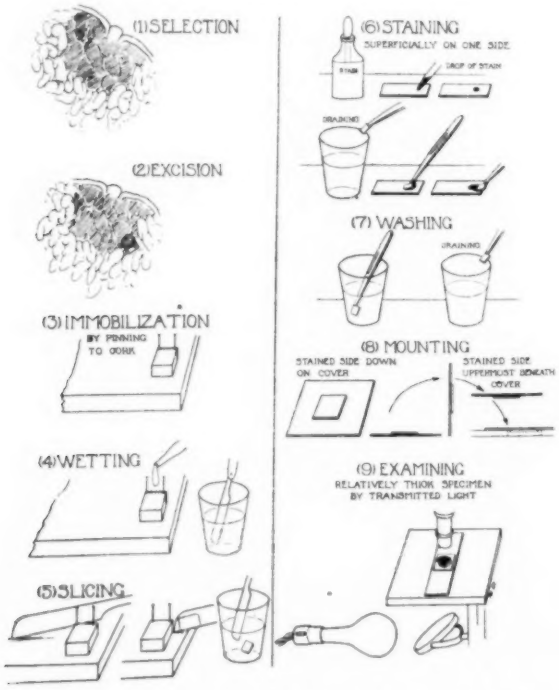

Fig. 2.—Nine steps in preparing the tissue for microscopic examination.

a tougher part, the latter should be pinned underneath. Otherwise the softer portion may be crushed when an attempt is made to cut through the tissue.

4. Wetting. In order to disturb the tissue cells as little as possible, just before slicing, both the razor and the tissue should be wet with distilled water, clear tap water or physiologic salt solution.* Wetting reduces friction and makes it easier to secure perfect sections.

5. Slicing. The object is to obtain one or more thin, smooth, plane-parallel slices which shall contain the area that one wishes to examine microscopically. Rest the point of the razor on the cork plate about 5 to 6 cm. to

*Theoretically physiologic salt solution is to be preferred, but in my experience the tap water in Nashville, Toledo, Chicago, Cleveland, Rochester and other cities, has given such excellent results that I usually use tap water or distilled water. The reader should try all three and choose the one that suits him best.

the left of the immobilized wet tissue. The point if properly placed is not raised again until the section is completely severed from the block. Before sectioning, the other end of the razor is raised and then lowered over the block so that by sighting over the blade one can see exactly how much tissue is to be cut off. *Do not press the razor into the tissue.* That would crush it. Instead, use it as if it were a very fine saw. Neither raising nor lowering the blade, draw it along the cork, using as near as possible the full length of the blade. In this way cut with one stroke completely through the tissue about 1 to 2 mm. in front of the supporting pins. If the razor is very sharp its own weight, as it is drawn along, will be almost enough to cause it to cut entirely through tissue of average resistance. The first piece cut off is usually discarded. Without changing the position of the razor in the hand, place the point once more on the cork to the left of the immobilized tissue and sighting over the blade to see how thin a piece you can cut, draw the wet razor once more through the tissue, this time securing a thin plane-parallel slice which can be stained at once or deposited in a small vessel containing distilled or tap water. In slicing, be careful not to bring the edge of the razor in contact with the pins. With a little practice you will be able to get surprisingly thin sections. But thin sectioning can be overdone. If the sections are too thin, they are harder to handle and before staining are best mounted on slides while they are still in water. Moreover, sections which in the beginning you are apt to regard as too thick, will probably give better results than you anticipate. If the amount of tissue is very small, every section cut should be stained and examined.

6. Staining. The stain should be applied superficially to one side only. There are several ways of doing this. The following is one of the best, and works well where the tissue is so thin that mounting is difficult. Place one end of a clean slide in the glass of water containing the sections and with forceps bring a thin, smooth, flat section in contact with the slide and pull both out of the water at the same time. This usually causes the section to spread out and lie flat on the slide. Without disturbing the section wipe off the excess of water on the slide. With a medicine dropper place on the slide near the section a small drop of good neutral polychrome methylene blue and spread it out thin. With forceps pick up one end of the section and raising it about 1 mm. drag it over on top of the stain and move it about gently for approximately 1 to 5 seconds. Carefully avoid getting stain between the prongs of the forceps or on both surfaces of the section. If this latter occurs, discard the section and try again. Unless the section is moved about, it may not stain uniformly, for the weight of the wet section may suffice to press out from beneath it most of the stain. The time required to stain varies with the strength of the stain, the amount of water dragged into it, and also with the avidity the cells have for the stain. If the section is not stained heavily enough the first time, it can be restained for a second or so. Thin sections are sometimes best stained by mounting them on a slide as already described, then after draining off the excess water, turn the slide over so that the section is below. With a fine camel's hair brush wet with the stain, the

under surface can be gently painted. Stain can also be applied to sections by glass rods or medicine droppers.

7. Washing. After staining, the section is picked up by one corner with forceps and is transferred to a glass of clean water where the excess of stain is gently but thoroughly washed off by moving the section up and down in the water three or more times. If the stain is painted on the section, it is sometimes best to wash it off with water spurted gently on it from a medicine dropper.

8. Mounting. The section should be mounted at once. If the section does not fold on itself when it is pulled out of the water, it can be drained and mounted by placing it stained side down on a clean cover. Press it gently into contact with the cover, turn the cover over, and the stained surface of the tissue will now be uppermost but adhering to the under side of the cover. In this position complete the mounting by placing the cover on a drop of water lying on a clean glass slide. Or if a slide with a hole in it is at hand, the cover may be so placed on this that the tissue lies in the hole. See illustrations.

If the stained tissue folds on itself when pulled out of the water, it can usually be easily mounted if, while it is still in the water, a cover glass is brought in contact with the stained side and both are pulled out together. In so doing the section should spread out smoothly and cling to the cover. Wipe off the water from the upper side of the cover and mount as before so that the tissue lies in the hole in the slide, stained side uppermost beneath a cover.

9. Examining. This is done at once by transmitted light. The light must be strong enough to transilluminate the tissue, but avoid using more light than you need. Adjust the mirror to get uniform illumination. Although the diaphragm may be left wide open, this is not advisable as light decolorizes the stain. It is best to cut the diaphragm down until a small illuminated circle is seen where the light strikes the tissue. Regulate the light by moving the mirror, and by lowering or raising the condenser of the microscope. If the section fades before the examination is completed, it can be restained one or more times. But the restaining cannot be repeated indefinitely, as the stain gradually sinks into the depths and then so alters the light transmitted through it that the specimen is no longer properly illuminated. By placing the stained tissue in alcohol, the stain can be completely removed. If the decolorized section is preserved in formalin, it can be restained whenever this is desired.

10. Diagnosing. The rapid microscopic diagnosis of razor sections is often very easy. Sometimes it is difficult. The harder it is, the more one should know about the tissue. Before attempting the rapid microscopic diagnosis of fresh tissue, the pathologist whenever possible should see and examine the tissue in the gross. To know the exact source of the specimen and its size, shape, color, and consistency is usually more important than to know the history of the case. Razor sections of fresh unfixed tissue, if properly cut and stained, resemble good frozen sections. Those trained to diagnose frozen sections of unfixed tissue similarly stained should, therefore, from the begin-

ning have no difficulty with razor sections of favorable tissues, such as most breast tumors. But pathologists experienced in the diagnosis of fixed tissue only, should, until they are thoroughly familiar with the appearance of unfixed tissue cells, control all of their razor section diagnoses by the method they know best.

NEW METHOD

In believing this method new I may be in error, although up to the present I have found nothing quite like it in the literature. The newness, however, does not lie in using a razor to section tissue. Before the discovery by Stilling,[8] Jan. 25, 1842, of the advantages of freezing tissue and before the introduction of microtomes by H. D. Schmidt,[9] Hensen,[10] His[11] (1866), Julius Cohnheim,[12] and others, the razor was frequently used for sectioning tissue. The older pathologists knew that unfixed tissue is often soft and difficult to cut. They tried various methods of immobilizing it, holding it between their fingers, embedding it in amyloid liver, in sliced elder pith, etc. Then they learned that fixed tissue is much easier to cut, and they became surprisingly skillful in making razor sections of this. Nevertheless, so far as I am aware, no one of them hit upon the method here suggested of staining thin plane-parallel razor sections of unfixed tissue very superficially on one side only, using a rapid vital stain, and examining the sections under glass by transmitted light. This seems to be new.

VALUE OF THE NEW METHOD

The new method has now been tested on over six hundred fresh unfixed tissues diagnosed malignant by the Pathologists of the Mayo Clinic as well as by me. In many cases the tissues were very easily diagnosed. Some tumors were more or less difficult, requiring a number of sections to be cut before the diagnosis could be made. A few in the fresh state were practically impossible of diagnosis. Up to the present, however, apparently no false positives have been given. The number of cases found to be easy increased as the technic improved. The accompanying table shows some of the things learned in examining the first six hundred malignant tissues.

In the table I have marked as more or less difficult all examinations in which I failed to record at the time that I regarded the diagnosis as easy. But my records of the ease or difficulty experienced in examining the first two hundred cases are somewhat incomplete due to interruptions caused by visitors. My statistics showing more or less difficulty in thirty-eight of the first hundred and in eighteen of the second hundred are for this reason not quite fair to the method. But I have let them stand as I cannot correct them accurately. Another thing that has reduced the percentage of cases easily diagnosed is the fact that occasionally the lesions were very small, and the part that I had for examination may not have contained any malignant areas.

The organ most frequently examined was the lymph node. The easiest was the breast. The most difficult tissues were small friable uterine scrapings and small soft papillomatous carcinomas. These constituted only a small part of the total number of tissues examined.

TABLE SHOWING THE RESULT IN EXAMINING 600 FRESH, UNFIXED TISSUES, GIVING TOTAL NUMBER AND ALSO INDICATING HOW MANY SPECIMENS IN EACH 100 EXAMINED WERE FOUND MORE OR LESS DIFFICULT

	1—100		101—200		201—300		301—400		401—500		501—600		SUMMARY 1—600	
	TOTAL	MORE OR LESS HARD	TOTAL	MORE OR LESS HARD	TOTAL	MORE OR LESS HARD	TOTAL	MORE OR LESS HARD	TOTAL	MORE OR LESS HARD	TOTAL	MORE OR LESS HARD	TOTAL	MORE OR LESS HARD
Lymph nodes	22	8	34	8	33	1	25	0	24	1	31	3	169	21
Breasts	23	5	11	0	21	0	15	2	21	0	17	0	109	7
Stomach	10	6	9	3	9	2	16	0	6	0	16	0	66	11
Cecum	4	2	2	0	1	0	4	0	3	0	3	0	17	2
Rectosigmoid	14	10	14	3	15	1	12	0	14	0	10	0	78	14
Miscellaneous	27	7	30	4	21	5	28	1	32	7	23	1	161	25
Total	100	38	100	18	100	9	100	3	100	8	100	4	600	80

A Comparison.—For over ten months razor sections and frozen sections of the same tissue have been compared. According to pathologists who have seen both, the razor sections in nearly every instance were faster, and in many cases were said to be better stained and easier to diagnose than the frozen sections.

Dependability.—Repeatedly microscopic diagnoses have been reported to the operating room by Drs. MacCarty, Broders, and Caylor on razor sections before the frozen sections were ready to be examined. Razor sections have been good enough for Dr. MacCarty[13, 14, 15] to make from them his cytologic diagnosis of malignancy. They have also enabled Dr. Broders[16, 17] to apply successfully his rules for grading malignancy.

Many doctors visiting the Mayo Clinic in the last few months have seen the new method and have been very enthusiastic about it. The qualities which seemingly have made the strongest appeal are its simplicity, inexpensiveness, rapidity, and dependability.

Tested by Others.—Under my direction a number of physicians have tested the technic, and on favorable tissue no one has failed. Most of the doctors made good sections at the first or second attempt.

ACKNOWLEDGMENTS

During the last eleven months I have been privileged to work as a guest in the Department of Surgical Pathology of the Mayo Clinic. Free access to an abundance of perfectly fresh material has been given me and everything possible done to facilitate my work.

Without the splendid spirit of helpful cooperation shown me, the work here reported would have been greatly delayed and might never have been done. For the material put at my disposal, for the interest taken in my work, and for the aid they have so freely given, I desire here to express to Drs. MacCarty, Broders, Caylor, and Owen my very deep appreciation and cordial thanks.

To Drs. Robertson and Mills I also gratefully acknowledge my indebtedness for many opportunities of examining autopsy material.

SUMMARY

The method of tissue examination described in this paper is apparently new, easy, inexpensive, and dependable. It is applicable to fresh unfixed tissue, and it permits the microscopic study of cells that are seemingly still alive. The technic for fresh tissue can be applied also to formalin fixed tissue. Artefacts are few. The method is so rapid that reliable microscopic diagnoses of tissue can often be given by an experienced pathologist in less than sixty seconds. While it is probably especially useful in the diagnosis of malignancy, it is not limited to this. It should be of value in studying and teaching cytology, histology, and pathology. It seems almost ideal for use in the doctor's office, in the operating room, in hospital laboratories, and, in an emergency, in the patient's home.

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DISCUSSION

Dr. Wm. Carpenter MacCarty.—I think I know perhaps as much about this method as anyone except Dr. Terry, and the best way to learn it is to go and see his demonstration. I have seen it almost daily for the last eight months and would be perfectly willing to take a bottle of his stain, a couple of pins and a razor, and make almost any diagnosis that I would make by any other method. I do not need the microtome. The microtome has the advantage, however, of making a section which will keep a whole day if you want to study it over a long period. So far as actual recognition of the things which are diagnostic I would as soon have Dr. Terry's preparation as any made with a freezing microtome. I would advise you to look into his method, for if you are interested in tissue you cannot afford to miss the opportunity. Dr. Terry is the first Professor of Pathology who has seen fit to spend time enough to investigate our fresh tissue methods and diagnosis. You cannot expect anyone to learn tissue work in a few months, and it is going to take brains to see and interpret the findings for practical purposes. Dr. Terry's method is of very great value not only to the practical tissue diagnostician but to teachers of pathology and histologic anatomy.

I know that in our clinic no clinician has a better diagnostic efficiency by clinical signs and symptoms than 70 per cent. There is probably not a gross pathologist who can diagose everything as it comes, in over 80 per cent of cases. In my own experience in a study of 42,000 specimens, gross diagnostic efficiency is not over 80 per cent. It must be remembered that no man can become a great gross diagnostician until he is a microscopic diagnostician. In all cases the pathologist should have access to the patient, as well as all clinical data and surgical findings.

Dr. Harold G. Palmer.—I have been tremendously interested in the matter of frozen sections for quite a number of years; as a matter of fact I did the first frozen section that was ever done in Rhode Island some twenty-eight years ago. From what I have learned tonight I feel that I am just in the kindergarten grade. I have been impressed with what Dr. Terry has shown today and what he has said and with the remarks that Dr. MacCarty has made tonight. This method of methylene blue staining with the fresh tissue brings out tremendous possibilities, it seems to me, but we must consider the fact that we have an entirely different angle from which we are looking at the picture. All of our pathology and histology has been passed on an interpretation of a compound structure, considering the morphology of cells and structures as they are arranged. As I understand from my talk with Dr. Terry today, with this method the interpretation is based on the study of only the nucleus and nucleoli of the stained cells. I said to Dr. Terry that I was interested; I should be glad to follow this through. I would also make my frozen sections and compare them side by side and study the different cells. I feel that it is a very dangerous procedure to go home and say this is easy; we can do this. You cannot go ahead and interpret soundly without having a great deal of experience. I feel that tissues should be submitted to men of

Dr. Terry's experience and be checked up on your findings. Another point which is of very vital importance to my mind is this matter of the technicians working on frozen sections and technicians' interpretation. Under no circumstances should technicians interpret on a tissue or any other laboratory procedure.

Their job is to prepare material for the pathologist to study, and when such preparation is completed, the technicians' obligation and responsibility terminate immediately.

Dr. Terry's rapid method for fresh and fixed tissues is a distinct addition to our laboratory technic. The frozen section with hematoxylin and eosin staining will continue to be a valuable procedure and under no circumstances should it be thrown into the discard.

Dr. Philip Hillkowitz.—May I discuss this presentation from a practical view? In the maze of details of technic of the microscopic examination one is apt to forget the value of gross appearances. In observing the trees, we lose sight of the forest. In most cases where an immediate diagnosis is necessary, during an operation, the trained pathologist is able to render correct opinion based on the naked eye picture. The hospital laboratory director should school his sense of sight and palpation so as to be able to recognize malignancy on inspection. I am greatly inclined to maintain the thesis that, given adequate experience and schooling, all tumors can be diagnosed by the naked eye. This is especially true in neoplasms of the breast. Unfortunately not all of us have the opportunities to see a large number of cases or the faculty of profiting by the observation. It is, however, an ideal that we should endeavor to attain. So with all due appreciation of the value of improved technical methods in extemporaneous frozen sections permit me to make a plea for greater attention to gross details.

ACCURACY AND PRECISION IN CLINICAL PATHOLOGY*†

By Philip V. Wells, D.Sc., Newark, N. J.

INTRODUCTION

IN ANY discussion of accuracy it would seem most appropriate to begin by emphasizing accuracy of statement, because no one who reads scientific literature can help being confused by the looseness and ambiguity generally associated with expressions concerning precision and accuracy. Such statements as "The measurement is good to 1 per cent," "The experimental error in no case can exceed 5 per cent," "The method is reliable to ½ per cent," etc., seem to many authors satisfactory descriptions of accuracy. When one faces the facts, however, such expressions are seen to be meaningless, and one wonders if the authors are always aware of this. Perhaps the labor involved in obtaining sufficient data to determine the precision of a particular method, let alone a significant estimate of its accuracy, is in part responsible for this situation.

This paper will be restricted to an effort to define in a practical way the significance of the terms precision and accuracy as they may be applied to clinical pathologic methods.

ERRORS AND REPRODUCIBILITY

An experimental method may be very precise but quite inaccurate. Precision is evident from the data, accuracy is always in doubt. There is a whole hierarchy of errors, each including those of lower order, and accuracy

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is the net result of them all. For example, a hemacytometer may have inaccurately ruled lines, which would give counts systematically high as long as it is used, but its cement might loosen after some months, thereafter reducing the counts systematically, and these two systematic errors might exactly compensate for a time to give correc results. The precision of the counting chamber, if investigated by repeating counts, would not change, because each count would be high by the same amount before the cement loosened, and low by a fixed amount thereafter. To reveal such inaccuracies, different chambers should be compared with each other on the same sample of blood, or better, the actual dimensions of the chamber should be measured. The precision of the blood count, on the other hand, might depend almost entirely upon the mixing in the pipette, or upon the perfection of the blood sampling.

The fluctuations arising from personal idiosynerasies of technic are usually fairly small, and can be largely eliminated by repetition of the measurement. To attain accuracy, however, both accidental and systematic errors must be reduced. Accuracy implies that a method be reproducible in its entirety and give absolute results, the same in every hospital laboratory throughout the country from one decade to the next, so that experience can accumulate on a fixed basis in sufficient volume to make scientific progress possible. The reagents, the instruments, and the criterion of measurement must all be reproducible from specification, or else continuity must be maintained by means of permanent standards. These requirements are, of course, to be interpreted reasonably, and an instrument need not be ten times as precise as the normal fluctuations in the samples measured. In pioneer work relative results often suffice, but for clinical interpretation methods should be standardized just as are the units of mass, length, and time.

When a particular method happens to be the only one available, differences in the results are apt to be ascribed entirely to variations in the samples, and a spurious specificity is often in this way attributed to the phenomena under study. Only those variations are significant which are not errors of measurement. How are we to distinguish such errors from real phenomena? As a matter of fact, we can never be certain that a given variation is not an error, but correct statistical methods will help us to estimate the chances in favor of one interpretation as compared with another.

AVERAGE VALUES: THE MEDIAN

Many errors arise from causes which are quite apparent. The complex interplay of known factors account for some, while the sources of other errors may be wholly unknown. Whatever the origin of the variations observed in a quantity which theoretically should be constant, they are usually lumped together and ascribed to accidental causes or to "chance." Chance variations cannot be predicted individually. In sufficiently large numbers, however, they are supposed to distribute themselves in a fixed manner on either side of the "true" value which they are assumed to represent. Upon the stability of such distributions of errors depends the actuality of what has been called the "true" value. This is a mean or average value the

choice of which is a matter of convention. Indeed, the choice of characteristic to be observed is quite arbitrary, a fact generally overlooked in statistical literature

It is customary to use the arithmetic mean obtained by adding all the observations together and dividing by their number. This simple average is regarded by some as theoretically the most fundamental, but might better be regarded as a simple approximation to the "median" or middle value. The median is defined as a value of such magnitude that it stands between the half of the observations which are less, and the half which are greater. The median was considered the "most advantageous" value by Laplace because it rendered the sum of the deviations (taken without regard to sign) a minimum. The arithmetic mean renders the sum of the squares of the deviations (from it) a minimum. Both are arbitrary. The most important advantage of the median is that *it is the only average which does not change with change of variable.*

In analyzing data it is often found that some function of the variable which was originally observed is related to other quantities more simply than is the original variable. For example, the square, or logarithm, might give a straight line when plotted against some other variable. But the square of the arithmetic mean is not the arithmetic mean of the squares of the observations, and the logarithm of the arithmetic mean is always larger than the arithmetic mean of the logarithms. Even the mode, or most frequent value, suffers from the same difficulty. The logarithm (or any other function) of the median, however, is the median of the logarithms (or any other function). This is because the median is defined by frequencies alone, the magnitude of the variation having no other influence than to place it in one of two classes, above or below the median. For the purposes of interpretation, therefore, the *median* is the best average.

In practice the median is simply and quickly determined. A glance at the figures shows about where they tend to cluster. The values falling in this neighborhood are arranged in order of magnitude, those falling above and below are simply counted. Thus, if there are twenty values in all, say six below those in order and nine above, the median falls between the fourth and the fifth values arranged in order. Usually both the fourth and the fifth will be equal, but if they differ some convention must be used to define the median. In most cases a value half way between the middle pair of values gives a good median when the number of observations is even. When the number is odd, there is but one middle value. Some statisticians use a "smoothed median" to decrease the fluctuations due to smallness of samples. One can, therefore, take the arithmetic mean of three or four middle values and thus avoid the overemphasis of the ordinary arithmetic mean on extreme values, and yet retain its smoothness even with small samples.

The essential characteristic of an average is its stability, which makes it more useful than any individual observation, because it represents all the data and is not dominated by a few. Now in any case where data vary widely, a few very large values, representing perhaps less than 1 per cent of the results, may halve or double the value of the arithmetic mean, but

their effect upon the median is slight. This renders the arithmetic mean practically valueless in distributions of wide variability.[1]

Everyone has experienced difficulty in deciding what to do with an extreme when only a few observations are available. If it is included, the arithmetic mean is probably biased toward it, and yet it seems arbitrary to discard the observation entirely. The smoothed median avoids any such bias because the middle values are the only magnitudes involved, and the extreme variations merely locate the middle values. It is unlikely that the extreme variations should have as much weight as the middle values, whereas the arithmetic mean gives them the most weight. The smoothed median is, therefore, the most appropriate average for small samples.

THE AVERAGE DEVIATION AS A MEASURE OF VARIATION

The simplest measure of the amount of variation among the observed values is the sum of the deviations from the median (taken without regard to sign) divided by their number. This is usually called the *average deviation*, and abbreviated *a.d.* Some statisticians call it the mean error, but others use this expression for the square root of the arithmetic mean of the deviations squared, which is generally called the standard deviation (σ). Squaring the deviations is a mathematical device for eliminating the negative sign. Moreover, the arithmetic mean of the observations is the average which renders the standard deviation (from it) a minimum, just as the average deviation taken from the median is a minimum.

For mathematical statistics the arithmetic mean and standard deviation are more convenient than the median and the average deviation, but in practice the latter are much easier to compute. Logically, the advantages are also with the average deviation, for the standard deviation gives altogether too much weight to extreme deviations, which are often mistakes of the observer, and without significance. With small samples, the difference between the values of the average and standard deviations is of no importance (except to emphasize the insufficiency of the data) so that it is pedantic to perform the extra work of squaring the deviations. To shorten the labor, the a.d. should not be computed from the exact value of the median, but from the nearest round number. It is useless to retain more than two figures in deviations, and one figure is often sufficient.

It is possible to compute the a.d. every time an average is taken, but, of course, it would be a waste of time. Nevertheless, it is impossible to interpret data correctly without an approximate knowledge of the a.d., for without it errors cannot be distinguished from the phenomena of interest. Therefore, it should be the first duty of the interpreter of data to learn the a.d.'s of the averages used.

The a.d. of a single observation from the mean is merely a measure of the precision of the particular observations included. If these constitute a sufficient sample of the universe under discussion, the a.d. from the mean is also a measure of the accuracy of the data. Unfortunately there is no royal

[1]This point is discussed in more detail in section 9 of a paper by P. V. Wells and W. F. Wells, Jour. Am. Water Works Assn., 1922, ix, 502-27.

road to accuracy. The best we can do is to try to find the possible causes of variation, and to test the possibilities experimentally. Now, every such test means a series of observations from which an a.d. can be computed, which is the proper measure of the precision indicated by the test. The systematic errors are shown by comparison of the averages characterizing the conditions in different tests with the same standard. When all sources of error have been eliminated as far as possible and the technic of the method decided upon, *the final accuracy of the method can be expressed by the average deviation of a single observation from the mean of a series which includes all the variations with the same frequency as is met in practice.*

This definition of accuracy assumes a complete knowledge of all the technical details involved in practice, but only the widest variations need challenge attention because each deviation is the net result of all the random forces at play, which tend to neutralize each other. Indeed, it is always found experimentally that large deviations occur much less frequently than small ones, also that the average deviation in one series of observations is about the same as the a.d. in a second set under similar conditions. It is this stability of the average deviation which makes it useful in forecasting the future.

CHANCE VARIATION

Suppose two samples show a difference. Is the difference real or due to experimental error? Such a question poses the fundamental problem of accuracy which is the determination of the chance of its being one or the other. The chance is to be expressed in terms of the average deviation of a single observation from the mean of a series in which every factor except the sample has been allowed to vary at random. If this series were extensive enough, a smooth frequency curve of the deviations could be drawn, with the magnitude of the deviation as abcissa, and the relative number of deviations of that magnitude, as ordinate. This curve would rise rapidly to a maximum at zero deviation, then fall again more or less rapidly. On the other hand, if the deviations were plotted one after another in the order observed, they would show no regularity, but would simply fluctuate above and below the zero line, as do winnings in games of chance. We cannot predict the next deviation, however extensive our experience, and so we say it occurs by chance. But the shape of the frequency curve can be predicted with considerable accuracy, for if another large number of observations be made and a frequency curve drawn, it will be of practically the same shape as the first. It is the reproducibility of the frequency curve of the deviations and the random order of their occurrence that are characteristic of chance variations.

Experience shows that more than one-half of the deviations are less, and less than one-half are usually greater than the average deviation. There is, therefore, somewhat less than one chance in two that the next deviation will exceed the a.d. in magnitude. Further, there is usually a tendency for the errors to group themselves symmetrically, so that the chances of negative and positive deviations of equal magnitude are equal. Two simple negative

exponential functions have been found to represent approximately the frequency distributions in many cases. One is appropriate to the arithmetic mean and standard deviation, while the other is appropriate to the median and the average deviation. The law of the standard deviation involves squares in the exponent, the law of the average deviation involves only the first power. Tchebycheff has deduced a criterion which states that a deviation greater than (T) times the standard deviation (σ) occurs less than once in (T^2) times. While this makes no assumption as to the form of the frequency curve, unfortunately wide deviations usually occur very much less frequently than this upper limit. The chances indicated by these three criteria are shown in Table I. They emphasize caution in asserting that a phenomenon is real without knowing the actual frequency curve.

TABLE I

FREQUENCY OF POSITIVE DEVIATIONS EXCEEDING $T\sigma$

T	TCHEBYCHEFF'S CRITERION	LAW OF STANDARD DEVIATION	LAW OF AVERAGE DEVIATION
	LESS THAN		
	ONE IN	ONE IN	ONE IN
1	2	6	8
2	8	43	34
3	18	743	139
4	32	31500	568
5	50	—	2347

LAW OF THE AVERAGE DEVIATION

Frequency of deviations exceeding ± x is equal to exp (−x/a.d.).

Deviation (± x/a.d.)									
1	2	3	4	5	6	7	8	9	10
Frequency: one in									
2.7	7.4	20	55	148	403	1096	2985	8130	22222

If all sources of error are properly represented in the average deviation, Tchebycheff's criterion is too conservative, while the normal law probably errs in the opposite direction. According to this law the standard deviation is 1.25 times the average deviation, while the law of the average deviation gives the factor 1.41.

It is customary to state the "probable error" (p.e.), or median error, without regard to sign instead of the a.d. or σ, after an average, to indicate its precision. The median error has the same advantages among errors as the median has among averages. The probable error of a single observation is divided by the square root of the number of observations to obtain the probable error of the average. If the normal law holds, the probable error of a single observation (p.e.) is given by p.e. = 0.845 a.d. = 0.6745σ, while the law of the average deviation gives p.e. = 0.693 a.d. = 0.49σ. One-half the deviations exceed the probable error in absolute magnitude. In view of the uncertainty, however, regarding the actual distribution of the deviations, there is little point in using the probable error. The average deviation of a single observation, with a statement of the number of observations averaged, is sufficient.

The true probable error of the average, that is, the median deviation of a series of averages upon the same sample is seldom as small as the probable error of a single observation divided by the square root of the number of observations, because of systematic errors. There is no doubt, however, that the precision of the average increases with the number of observations included, but the returns of the extra labor diminish very rapidly.

Similarly, the rule that the resultant error is the square root of the sum of the squares of the component errors often fails quantitatively because these errors are not entirely independent of each other, as assumed in deriving the rule. Nevertheless, the conclusion that only the major sources of error need be considered, is amply justified by experience.

ACCURACY OF THE DUBOSCQ COLORIMETER

To illustrate the above discussion, some data are presented indicating the accuracy of measurements with the Duboscq colorimeter. The photometric field of the instrument used had a dividing line which practically disappeared when matched, so that the precision of the color comparison is probably better than that of the average Duboscq. Table II gives the results of a comparison of solutions of cuprammonium sulphate, the concentration in one cup being always twice that in the other. If this solution obeys Beer's law, the observed depths should be in the same ratio as the dilutions (reciprocal concentrations), and the data show no systematic departure from this dilution law over a 64-fold range in dilution. The last comparison is in error because the solution was too dilute to give enough color. To increase the color density of the blue copper solutions, and limit the spectral range, a monochromatic red filter was tried, but the improvement was not as much as expected.

The average deviation of a single observation is less than 1 per cent, so that the average depth ratios should agree still better with the dilution ratios. The actual errors, however, are more than 3 per cent. Tchebycheff's criterion would indicate that there is less than one chance in fifteen that these departures are experimental errors, and yet there is no reason to sus-

TABLE II

DILUTION LAW ON DUBOSCQ COLORIMETER (B. AND L. MICRO MODEL)
UNIT DILUTION = 5% $CuSO_4$.$4NH_3$ .H_2O

DILUTIONS COMPARED		OBSERVED DEPTHS				DEPTH RATIOS		DILUTION RATIO	ERROR	
		WHITE		RED*		WHITE	RED		WHITE	RED
LEFT	RIGHT	LEFT	RIGHT	LEFT	RIGHT				PER CENT	PER CENT
1	1	10.0	9.9	2.0	3.9	0.99	1.95	1	−1.0	−2.5
1	2	10.0	20.7	2.5	4.7	2.07	1.88	2	3.5	−6.0
4	2	30.0	14.5	10.4	5.0	0.483	0.481	0.5	−3.4	−3.8
4	8	14.1	30.0	5.0	10.0	2.13	2.00	2	6.5	0
16	8	30.0	14.8	30.0	14.9	0.493	0.497	0.5	−1.4	−0.6
16	32	14.6	30.0	14.5	30.0	2.15	2.07	2	7.8	3.5
64	32	30.0	15.4	30.0	16.2	0.513	0.54	0.5	2.6	8.0
64	128	12.2	30.0	12.9	30.0	2.46	2.33	2	23.0	16.5

	White	*Red*
Average error (dilution ratio = 2)	= +5.9%	−0.2%
Average error (dilution ratio = 0.5)	= −0.7%	+1.2%
Average error without regard to sign	= 3.8%	3.5%
A.d. of single observation	= 0.8%	0.3%

*Red: Wratten 71 red filter over eyepiece. White: No filter over eyepiece.

TABLE III

CONCENTRATION LAW ON B. AND L. DUBOSCQ COLORIMETER
BENEDICT PICRIC ACID SUGAR TECHNIC
KNOWN GLUCOSE CONCENTRATIONS COMPARED WITH 1 MG. STANDARD

CONCENTRATION (MG./C.C.)	OBSERVED DEPTHS		DEPTH RATIOS		PER CENT ERROR		DIFFERENCE
	SERIES 1	SERIES 2	SERIES 1	SERIES 2	SERIES 1	SERIES 2	
Standard at 10 mm. depth							
0.2	55.0	61.0	0.182	0.164	− 9	−18	9
0.3	40.6	45.0	0.246	0.222	−18	−26	8
0.4	26.2	25.6	0.383	0.391	− 4	− 2	−2
Standard at 20 mm. depth							
0.5	47.3	53.6	0.423	0.373	−15	−26	11
0.75	29.0	30.4	0.690	0.658	− 8	−12	4
1.0	20.0	20.1	1.000	0.995	0	0	0
Standard at 70 mm. depth							
1.5	40.2	51.7	1.74	1.35	16	−10	26
2	33.4	31.0	2.09	2.26	4	13	−9
2.5	27.6	25.6	2.54	2.73	2	9	−7
3	26.6	25.2	2.63	2.78	−12	− 7	−5
4	19.7	18.6	3.55	3.76	−11	− 6	−5
5	17.2	17.6	4.07	3.98	−19	−20	1
6	13.5	13.8	5.18	5.08	−14	−15	1
7	12.2	12.4	5.74	5.64	−18	−19	1
8	10.7	11.0	6.54	6.36	−18	−21	3
10	9.4	9.7	7.45	7.22	−26	−28	2
20	5.9	6.6	11.9	10.6	−40	−47	7
40	5.5	6.4	12.7	10.9	−68	−73	5
80	5.7	6.0	12.3	11.7	−85	−85	0
	Arithmetic mean error (conc. 0.3-3 mg)		=		− 4%	− 7%	3
	Average error (without regard to sign)		=		± 9%	±12%	8
	A.d. single observation		=		0.5%		

pect the accuracy of the dilutions, for every care was taken to make them exact. There is evidently some tendency to bias in color matching which makes the results less accurate than is indicated by the reproducibility of consecutive readings. There is no indication that the illumination (from a Mazda white lamp) was not perfectly balanced, but the fact that the errors tend to be positive when the more concentrated solution is in the left-hand cup, and conversely, shows a slight failure of the instrument to give ratios independent of depth. On the whole, the data indicate that the average error in comparing such solutions in the Duboscq at concentrations above 0.1 per cent will be less than 4 per cent.

This prediction is tested on the data in Table III, comparing the amber colors developed in glucose solutions of known concentrations by the Benedict picric acid technic. Two series of samples are compared, to indicate the reproducibility of the method. The average difference between the two series in the concentration range from one-third to three times the standard is 8 per cent, which is not much less than the average errors, 9 per cent in Series 1, and 12 per cent in Series 2. Since both standard and samples for this test were diluted with particular care from a single stock solution, there is no reason to suspect the concentrations, so that the increase in the average error must be due to systematic error. This is shown by the algebraic signs of the errors themselves. The arithmetic mean difference between two series is 3 per cent, which checks with the prediction from Table II.

TABLE IV

EFFECT OF DILUTION AFTER REACTION ON PICRIC-SUGAR TECHNIC
1 MG. STANDARD AT 20 MM. DEPTH
SAMPLE OR STANDARD DILUTED TO BRING DEPTHS BETWEEN 10 AND 30 MM.
COMPARED ON B. AND L. MICROCOLORIMETER BY A DIFFERENT OBSERVER FROM TABLE III

CONCENTRATION (MG./C.C.)	DEPTH RATIO	ERROR PER CENT	ERROR (TABLE III)
0.1	0.203	103	
0.2	0.306	53	−14
0.3	0.378	26	−22
0.4	0.47	18	− 3
0.5	0.55	10	−20
0.6	0.66	10	
0.7	0.725	4	
0.75	–	–	−10
0.8	0.84	5	
0.9	0.93	3	
1.0	1.02	2	0
3.0	2.65	−12	−10
3.5	3.02	−14	
4.0	3.56	−11	− 8
4.5	3.75	−17	
5.0	4.0	−20	−20
Average error (without regard to sign) (Conc. 0.3-3)		=	±10.0%
A.d. single observation		=	1.1%
A.d. (omitting one observation in 46)		=	0.6%
Median deviation		=	0.6%

The effect of dilution with water after the reaction, to bring the colors within close range when the standard is kept at a depth of 20 mm., is shown in Table IV. This series of concentrations of a glucose solution was made just as in the experiments of Table III, but was compared on the micro-Duboscq of Table II. The color matches in these studies were made by three observers: those in Table IV by Dr. Rose, in Table III by Dr. Schattner, and I made those in Table II. The errors are negative for concentrations greater than the standard in both Tables III and IV. When the sample is more dilute than the standard, the errors are negative in Table III but positive in Table IV. If the systematic errors are ascribed to the samples, the only difference in the technic is in the process of dilution after the reaction. Such complications, due to changes in the samples measured, illustrate the importance of standards in studying the accuracy of a method. At the bottom of Table IV is a typical example of the tendency of the average deviation to overemphasize extreme errors. A single observation has vitiated the a.d. Omitting this one reduces the a.d. by about one-half, to 0.6 per cent. This is evidently the better value, for it agrees exactly with the median deviation. But while both instruments give color comparisons quite up to the best standards of photometry in precision (a.d. ½ per cent), colorimetric methods as a whole may give average errors ten times as great. Within the usual range of concentrations, one-third to three times that of the standard, the average error of the Benedict picric acid technic for sugar is not much better than 10 per cent of the concentration.

It is evident from these examples that the accuracy of a method should be expressed in terms of averages. The tendency to mention the range, or

extreme error difference is to be deplored. The range is the most unreliable measure possible and seldom has any important significance. In practice, extreme results are eliminated by repeating the test which leads to the average deviation as a measure of precision. Range depends upon the number of observations. As these increase, the more chance there is not only of a wider range, but also of a freak result of no importance, which spoils the range. It is therefore not the range but the average deviation which repetition stabilizes.

SUMMARY

Correct statistical methods help us to estimate the chance that a variation is real and not experimental error, and the only variations which can be considered significant are those which are not errors of measurement.

Preference is given the *median* because it is the only average which does not change with change of variable.

With small samples the arithmetic mean of three or four middle values gives a smoothed median.

When all possible sources of error have been eliminated, the final accuracy can be expressed by the average of a sample typical of all the errors of the method.

The average error, not the range of the errors in any sample, gives the best expression for accuracy.

Data obtained with the Duboscq colorimeter are given to illustrate above.

DISCUSSION

Dr. Herman Sharlit.—This matter has always appeared to me to be very important. I wonder why doctors do not take the trouble to do it. Dr. Wells pointed out to us the different forms of determining the reliability of that average, the probable errors, standard deviations, and square deviations. We also should consider, before we make any manipulations, exactly how many values we have secured. I have always felt that in using the series of observations, probably the most reliable figures for this purpose would be an average of the middle three. This should be most significant. In biologic phenomena the number of observations made are usually very small compared to the amount that would be necessary for a larger figure.

Dr. Wells (closing).—The smoothed median is important, as Dr. Sharlit suggests, and it is popular among economic statisticians. Averaging the three or four middle figures gives the advantages of the median for a small number of observations. I might also point out that it is often an advantage to express differences in terms of percentage, which is more significant than are the differences themselves. In making tabulations I notice that there seems to be a tendency to compare each series of figures and to say that the variations are about the same, when the simple calculation of the average deviation would make the comparison quantitative.

SYSTEMATIC SEARCH FOR PATHOGENIC INTESTINAL ORGANISMS IN DISCHARGES OF HEALTHY AND SICK INDIVIDUALS*

By Lewis B. Bibb, Captain, Medical Corps, United States Army

THE occurrence of mobile colonies which progress through liquid media at a uniform and characteristic rate of speed has been reported in a previous paper.[1] The characteristic rate of progression, taken in conjunction with the reaction of the given organism toward lactose, furnishes a criterion for recognizing identity. With this object in view, a semifluid sugar[2] media has been devised for differentiating picked colonies of intestinal organisms. This paper is a report of further experiences with this semifluid "motility" media, which was used for subculture of 2521 additional colonies, picked from 795 Endo plates inoculated with feces of 505 normal and abnormal individuals.

During this tryout of the motility media, an attempt was made to bring about "enrichment" or overgrowth of the pathogenic organisms at the expense of other organisms. This attempt was a failure. It was found, however, that a 25 per cent solution of glycerin in broth would protect pathogenic organisms against overgrowth by others.

A contrivance was devised for this purpose of facilitating the growth of many discrete colonies on plates. This in turn led to a handy method of performing the agglutination test. And so this paper is a report on a method of differentiating the intestinal organisms systematically and with a minimum expenditure of time.

The specimens examined were principally feces and urine submitted by cooks, ward attendants, and other soldiers functioning as foodhandlers in the army. Specimens from some hospital patients were also submitted to test.

As was to be expected, colon and other bronzing organisms tended to overgrow the typhoid organism. In the attempt to favor the growth of the latter, various proportions of brilliant green were added to broth and to bile media. A concentration of brilliant green was found which would enable the typhoid organism to overgrow the colon organism, but no mixture of brilliant green was ever found which could favor the growth of typhoid, paratyphoid and dysentery organisms at the expense of nonpathogenic bacteria. In like manner citrated caffeine was tried in agar plates. This reagent inhibited all organisms except paratyphoid "B," and, therefore, was of little value. The attempt to find a selective media was then given up.

Instead, another plan was tried, according to which reliance was placed on a growth restraining reagent, namely, glycerin. It was found that mixed cultures emulsified in 25 per cent of glycerin retained their original relative proportions for several days. By growing and picking a greater number of colonies, pathogenic organisms could be detected with a fair degree of facility.

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The spray method of inoculating plates can be made to yield under optimum conditions about 1000 colonies per plate of 95 mm. diameter, in practice, one-fourth as many is the average. A pipette was devised (Fig. 1) which was simple, effective, and easily cleansed. This pipette yields a satisfactory cloud of fine misty spray. Fecal emulsions were filtered through paper before spraying in order to secure a fine dispersion of bacteria and thus promote the formation of pure rather than mixed colonies. From time to time plates were sprayed with pure or mixed stock organisms in order to test the technic. To prevent danger, the spraying was done in a large metal can in which was kept a layer of 5 per cent cresol solution. A fenestrated tin shield was placed on the plate at the time of spraying, to prevent contamination of the edges of the plate. Upon Endo plates inoculated with fecal emul-

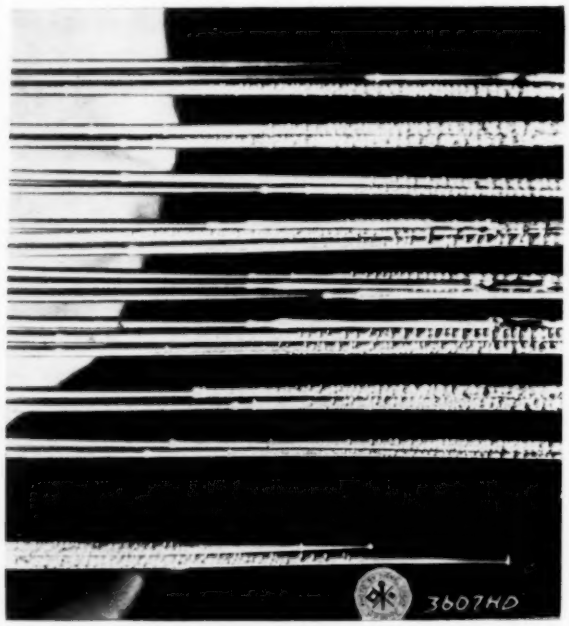

Fig. 1.—Gross appearance of organisms grown for nine hours in semifluid "motility" medium. All organisms are proceeding toward the left except those in the two lowest tubes. Top tube contains sterile medium. Tube next to the top, Eberthella typhi, shows one mobile colony. All remaining cultures are gas formers. Two mobile colonies proceeding "tandem-fashion" are seen in each of the following tubes; Nos. 4, 5, 7, 11, 16, 17, 18, 19, 20, and 21. (Photo by Signal Corps, U. S. Army.)

sions by the spray method, the average number of discrete pickable colonies obtained was about 225 per plate. The bronzed colonies outnumbered the nonbronzed in the ratio of about five to one. Levine's phosphate Endo containing no meat extract was used. This media is quite delicate, and gives picturesque results, especially when it is twenty-four hours old at the time of inoculation. Harris[3] formula was found highly satisfactory in making the media.

After twenty-four hours, colonies were picked to semifluid motility media containing lactose and saccharose. This media when contained in slender tubes as pictured in accompanying illustrations (Figs. 2 and 3) reveals

TABLE I

SPEED OF PROGRESSION, FERMENTING POWER, AND AGGLUTINABILITY OF INTESTINAL ORGANISMS

	GAS.	NO GAS.	TESTED FOR AGGLUTINATION	
			AGGLUTINABILITY	POSITIVE
Progression				
Less than 1 inch	225	310	Records incomplete	None
" 1 to 4 inches	801	69	69	20
" more than 4 inches	1088	28	28	2
TOTAL	2114	407		22
(101 colonies failed to grow)				

motility, speed of progression, and capacity to form gas from the contained sugars. It was found that of 2521 colonies picked, including typhoid colonies, 97 were motile and failed to produce gas, thus suggesting the typhoid and paratyphoid organisms. These suspicious organisms were all tried for agglu-

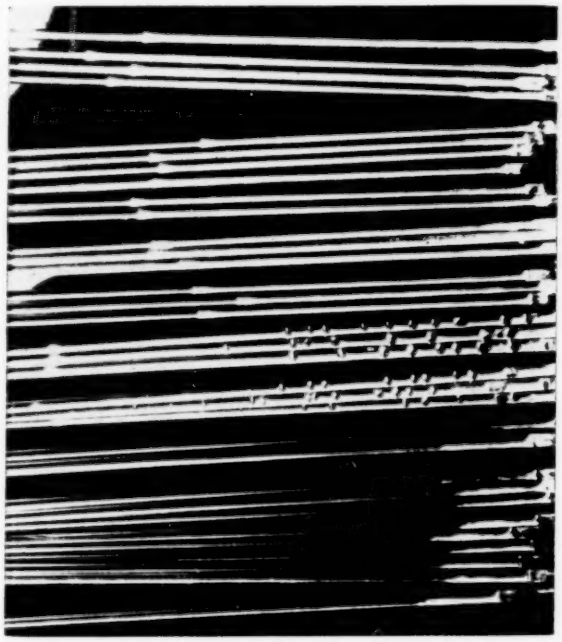

Fig. 2.—Stock organisms grown in motility media for nine hours, and proceeding from right to left. Beginning at the top: Tubes 1 to 4 contain Eberthella typhi. Tubes 5 to 10, Salmonella paratyphi. Tubes 11 to 16, Salmonella Schottmuelleri. Tubes 17 to 22, a gas former, probably Escherichia coli. All eleven remaining tubes below contain nonmotile organisms, Eberthella paradysenteriae or species of staphylococcus. All are inoculated to a depth of one inch in the tube. Nonmotile organisms have not progressed. Each tube containing motile organisms shows a hazy band at the most advanced (farthest left) portion of growth. (Photo by Signal Corps, U. S. Army.)

tinability by the method and with the results described below. Table I shows the classification of the organisms subcultured with special reference to their motility, their capacity to form gas in the media employed, and their agglutinability. The motility media indicated whether a given colony should be tried against immune serum of the nonmotile group (dysentery) or against that of the motile group (typhoid and paratyphoid). Table I shows that

about 15 per cent of all colonies grown in motility media required tests for agglutinability, but the bulk of these were nonmotile and, hence, required to be tried only against the antidysentery immune sera.

This relatively high number of subcultures requiring agglutination led to a method of agglutination by inoculating the organism into a broth containing a small proportion of homologous or polyvalent immune serum.

Numerous authors have reported growth of bacteria in homologous immune sera. Stryker[4] enumerates a dozen authors who have observed the behavior of various organisms principally those of the intestinal group under such conditions, and some observers report a loss of agglutinability by typhoid bacilli so grown. Stryker grew pneumococci in broth containing homologous immune serum, and found that prompt agglutination occurred when pneumococci were subcultured in immune serum for the first time.

The following experiments served as a basis for the agglutination technic described below.

It was found that typhoid organisms inoculated into plain broth containing 0.3 per cent antityphoid immune serum (of a titer of 1-3200) would grow in agglutinated form, showing as fine white granules suspended in practically clear broth. Inoculated into one end of a glass tube ⅛ inch by 36

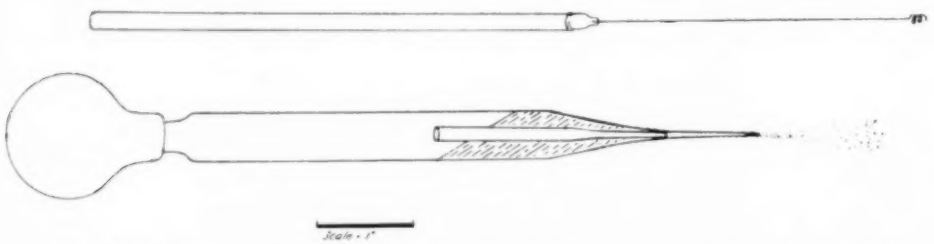

Fig. 3.—Spraying pipette, consisting of rubber bulb, large outer tube, and small inner tube. Inner tube ends at point where it touches outer tube. Large tube extends on to the end. Fluid level indicates angle in which pipette is held during use. Above, spiral wire for introducing immune serum into broth tubes.

inches filled with the immune serum broth, the typhoid organisms continued to progress for several months at the rate of about one inch a week. They were grown in the horizontal position at room temperature. The agglutinated masses could not be broken up by agitating the tube. If a similar tube were filled throughout one-half its extent with plain broth and throughout the remaining half with the immune serum broth as described, the organisms introduced would progress several inches a day (more rapidly than in motility media) while traversing the plain broth, but when encountering the immune serum would form the white granules mentioned above and would slow down their pace. The gross appearance of the organisms underwent a regular and definite change at or near the point where they first encountered the immune broth. In the plain broth they left behind them a narrow streak. As they encountered the immune broth they formed a broader granular streak. The granulations constituting a positive agglutination test can be seen at the end of twenty-four hours in favorable instances, but they are larger and more striking after forty-eight hours. One strain of typhoid organisms was encoun-

tered which did not agglutinate satisfactorily by this technic; namely, the "Rawlin's" strain. Once formed, the agglutinated masses persisted for a year and then gradually disintegrated. Cultures in broth without immune serum remained unagglutinated for two years.

It was found possible to mix the sera corresponding to the three organisms constituting the typhoid, paratyphoid group without losing group specificity. The method of agglutination adopted for routine use was as follows: The mixed "TAB" serum (consisting of antityphoid, antipara *A*, and antipara *B* sera) was kept on ice and just before use was added to the broth contained in slender tubes. For transferring and mixing the droplet of serum, a nichrome wire was wound into three spiral turns at the tip, since by actual trial this yielded a droplet of serum which was effective in the tubes used. (Fig. 1.) It was found difficult to get a satisfactory technic for tubing the immune serum broth until the nichrome spiral was invented. Finally the tubes were inoculated with the organism to be tested. These tubes were about $\frac{1}{8}$ inch by 10 inches in size. The serum was introduced and mixed by the nichrome spiral to a depth of four inches. The inoculum was thrust in to a depth of six inches. The tubes were then incubated at 37° C. for twenty-four hours. Known typhoid or paratyphoid organisms gave typical agglutination as described above. One per cent xylose was added to the broth used in these agglutination tubes. In positive cases, paratyphoid "B" organisms produced gas, while the typhoid and paratyphoid "A" organisms formed no gas. It was borne in mind that the differentiation of the pathogenic organisms one from another is relatively simple, while the first separation of the pathogenic from the non-pathogenic organisms is difficult. It was found that the agglutination method was rapid; it enabled us to test each suspicious colony against all three of the motile pathogenic organisms simultaneously. The possibility of infection from the living organisms was reduced to a minimum, and the tube was of value because it could be preserved as a record and compared with the others. Apparently, organisms which grow in clumps stand out more clearly than organisms which form clumps after having come into existence in dispersed form. It is to be noted that when immune serum is added to one end of a slender tube which is then inoculated as described, the organism comes in contact with virtually all possible dilutions of serum.

A mixed dysentery serum was satisfactory against all but the Shiga organism. These tests, however, were not carried on as consistently as in the case of the typhoid paratyphoid group, and a high titer antidysentery serum of Shiga type could not be obtained.

Practically no bacillus carriers were found. One colony was found which gave the ordinary tests characteristic of the paratyphoid "A" organism, but repeated examinations failed to reveal any further colonies of this organism. Likewise, one colony gave the ordinary tests characteristic of the paratyphoid "B" organism, but it could not be found again. No typhoid organisms were found except in two cases of almost typical typhoid fever. These two cases furnished 15 plates bearing 9000 colonies, of which 844 were regarded as

suspicious. Of the suspicious colonies, 132 were subcultured in motility tubes and 53 appeared suspicious. Of the 53 tested for agglutinability, 20 were positively agglutinated.

For encouraging the study, thanks are due Col. E. R. Schreiner, M.C., Commanding Officer, Tripler General Hospital. For routine assistance, acknowledgments are due Privates First Class Victor E. Del Vecchio and Thomas J. Costic, Medical Department, United States Army.

SUMMARY AND CONCLUSIONS

1. A serviceable pipette has been devised and described, which is suitable for inoculating plates by spray method, thus yielding many discrete colonies.

2. The behavior of an organism when grown in motility media determines whether it should be tried against agglutinating serum of the motile group (typhoid and paratyphoid) or against that of the nonmotile group (dysentery); or whether test for agglutinability may safely be omitted.

3. A safe and handy method of performing the agglutination test is described. This test yields white granules which preserve their form for months.

4. No carriers were found among the 505 soldiers examined. A single colony of each of the paratyphoids was seen, but these failed of confirmation on further, oft repeated examinations.

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SIMPLIFICATION OF THE TECHNIC FOR THE WASSERMANN TEST

By Leon H. Cornwall, M.D., Desiderius Groszberg, M.D., and Blanche C. Taylor, New York City

A SEMIAUTOMATIC distributing apparatus designed to facilitate the operation of pipetting was described by Cornwall and Schmitt in 1922.[1] Since that time it has been in constant use in the Pathologic Laboratory of the New York City Hospital, and as a time saver it has earned for itself the respect of our laboratory staff.

It occurred to one of us (D. G.) that further conservation of time could be realized in the performance of the Wassermann test by making three preliminary mixtures of the ingredients and adding more than one ingredient at a time. The question immediately arose concernng the desirability of mixing antigen and complement and then adding this to blood serum. Data were available concerning the effect of adding serum to a mixture of complement and antigen after the latter had stood for a period of one-half hour. Kolmer's[2] experiments on this point showed that there was a slight increase in

the fixation of complement when this method was compared with the successive addition of serum, antigen, and complement. In Kolmer's experiment with eighteen syphilitic sera tested in five doses, there was no difference in the fixation of complement in seven sera. In three sera there was very slightly more fixation with the method of successive addition, and in eight sera there was slightly more fixation with the method in which antigen and complement was allowed to stand for one-half hour before the addition of serum.

For our purposes we did not contemplate allowing the mixture of antigen and complement to stand longer than the time that would be required to add it to the blood sera by means of the "distributor." In view of the fact that, by means of this apparatus, the mixture could be added to sixty tubes a minute or to three hundred tubes in five minutes, we felt certain that this technical alteration would not modify our results to any appreciable extent.

In the same experiment Kolmer's results also indicate a slight increase in the fixation of complement when serum and antigen are allowed to be in contact for one-half hour before the addition of complement. With the method of pipetting by hand we could easily confirm the fact that, with our usual number of seventy-five to one hundred tests, the interval during which the complement and antigen were in contact before the addition of the mixture to the sera was much less than the interval during which sera and antigen were in contact before the addition of complement by our usual technic.

Before utilizing the new method as a routine procedure, however, we compared 416 sera with the old and the new technic. Among this number there were differences in twelve cases, the modified technic being more sensitive. An investigation of those twelve divergencies revealed that eight sera from cases of known syphilis gave slightly stronger reactions by the new method. Three sera gave negative reactions with the old technic and two plus fixations with the new. In the case of two of these there were histories of miscarriages, and in the third syphilis was denied. In one case no history was obtainable.

The routine technic for the Wassermann test that was used in the Pathologic Laboratory of the New York City Hospital before the modification described herein accords with a description published several years ago,[3] with the exception of a few minor modifications.

Volume.—This is 1.0 c.c. or one-fifth of the original Wassermann-Citron amount.

Antigens.—These consist of a cholesterinized and crude alcoholic extract. The cholesterinized antigen is employed in from five to ten units but never in excess of one-fifth of the anticomplementary dose. The alcoholic antigen is employed in two or more units but never in excess of one-half of the anticomplementary dose.

Incubation.—Fifty minutes at 38° C. for cholesterinized antigen and four hours at from 0° to 8° C. for crude alcoholic extract.

Hemolytic System.—Sheep cells 5 per cent and antisheep rabbit immune serum. Cells are sensitized by mixing with amboceptor and allowing to remain at 38° C. for one-half hour before use.

Hemolytic Titration.—Complement is titrated against two units of amboceptor, and two units of complement are used in the test.

Two sets of reactions of three tubes each are set up; one for the water-bath and one for the ice box.

This "set up" applies to both cholesterinized and crude alcoholic antigen sets.

The ingredients with the exception of blood serum were pipetted formerly in the order shown in the "set up." The serum was pipetted in the usual manner. The antigen, complement, and saline solution were added

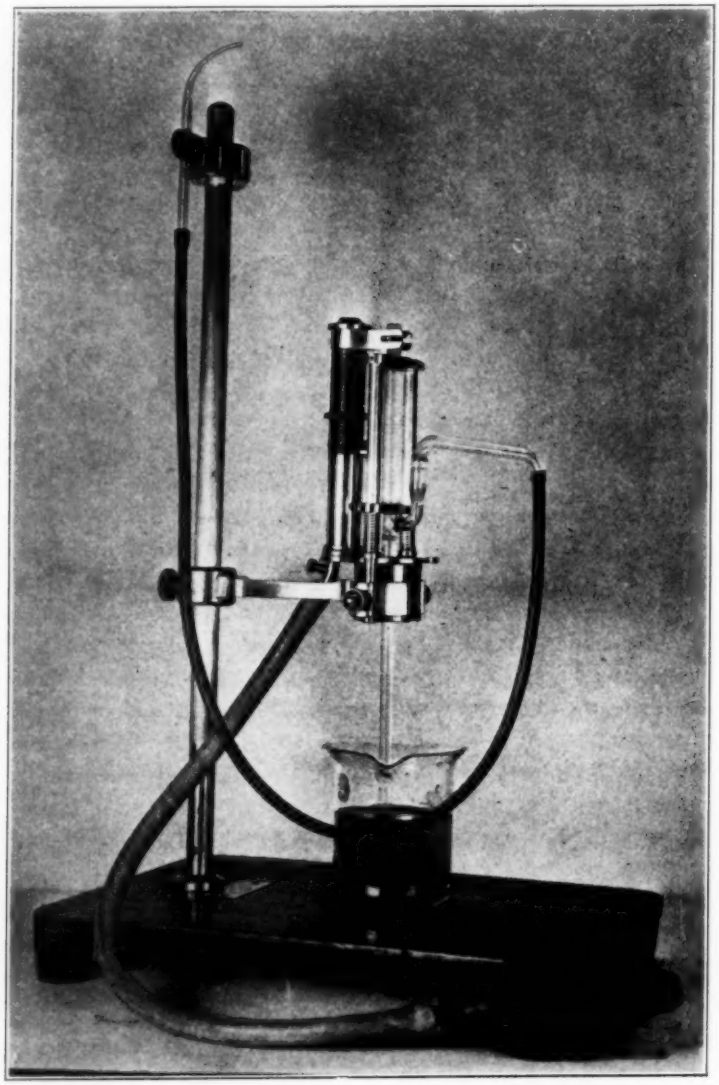

Fig. 1.—Semiautomatic distributor.

separately with the distributor. (Fig. 1.) After preliminary incubation of serum, antigen, and complement, the sensitized cell-amboceptor mixture was added.

The following description will indicate the manner in which the distributor was used formerly. It was first adjusted to deliver 0.1 c.c., and with this adjustment the crude alcoholic antigen was distributed to the set for the

TABLE I

SAMPLE SET UP FOR BLOOD

		I c.c.	II c.c.	III c.c.
Serum		0.04	0.02	0.04
Antigen dilution		0.2	0.1	0.0
Saline solution		0.16	0.28	0.36
Complement dilution		0.2	0.2	0.2
Amboceptor dilution Sheep cells 5%	Sensitized	0.4	0.4	0.4
		1.0	1.0	1.0

ice box. Two deliveries were made to each first tube (0.2 c.c.) and one to each second tube (0.1 c.c.). The distributor was washed with saline solution and the cholesterinized antigen was distributed in the same manner to the water-bath set. The adjustment was then changed to 0.12 c.c., and after washing out the distributor, this amount of saline solution was distributed to the second tubes. It was then changed to 0.16 c.c., and this amount of saline solution was distributed to all tubes. It was changed again to 0.2 c.c., and this amount of saline solution was distributed to the third tubes. This

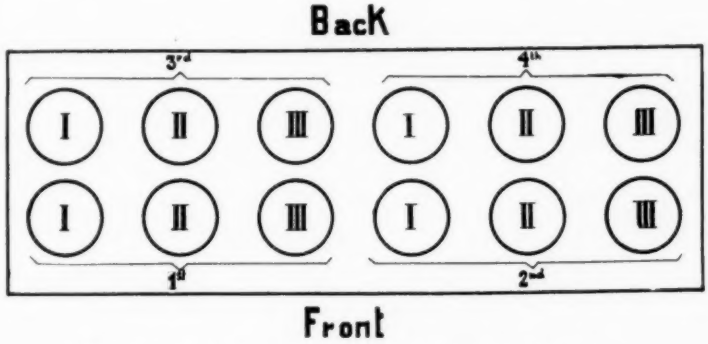

Fig. 2.—The "set up" of one rack carrying four tests.

gave a distribution of saline solution as follows: first tube 0.16 c.c., second tube 0.28 c.c., and third tube 0.36 c.c. Using the same adjustment, complement was added to all tubes. The distributor was again washed with saline solution, and the adjustment was then changed to 0.4 c.c. After preliminary incubation sensitized cell-amboceptor mixture was added to all tubes.

This procedure required eight pipetting operations as follows: (1) serum, (2) alcoholic antigen, (3) cholesterinized antigen, (4, 5, and 6) saline solution additions, (7) complement, and (8) sensitized cell-amboceptor mixture. The distributor was washed three times: first after the addition of crude alcoholic antigen; second after the addition of cholesterinized antigen, and third after the addition of complement.

TECHNICAL MODIFICATIONS

We shall now describe a modification in the technical procedures concerned with the distribution of the several ingredients which economizes time and, in our opinion, conduces to precision.

ARRANGEMENT OF TUBES

The three tubes for each antigen set are arranged in a row. We use racks that have two rows of six tubes each. Both front and back rows are used. Each rack, therefore, serves for four tests (Fig. 2). The racks are arranged on the table one behind the other.

SERUM

A set of empty tubes is arranged and serially numbered corresponding to the tubes containing the sera to be tested. Into each tube 2.0 c.c. of saline solution are pipetted and also 0.5 c.c. of inactivated serum. The contents are thoroughly mixed, and the pipettes are left in the tubes after the addition of serum. Two-tenths c.c. of the diluted serum is added to the first and third tubes and 0.1 c.c. to the second tubes.

When the amount of serum is insufficient to secure 0.5 c.c. for the dilution, smaller amounts, of course, may be used. The object of diluting the serum is to obviate the necessity of pipetting small amounts, thus diminishing the possibility of small but uncontrollable errors. This feature is in accord with that utilized by Kolmer.[4]

Next, three mixtures of antigen, complement, and saline solution are prepared, the total quantity of each depending upon the number of reactions that are to be performed.

PRELIMINARY MIXTURES

Mixture No. 1 consists of:

Antigen	(diluted to the proper titer)	1 part
Complement	(diluted to the proper titer)	1 part

Mixture No. 2 consists of:

Antigen	(diluted to the proper titer)	1 part
Complement	(diluted to the proper titer)	2 parts
Saline solution		2 parts

Mixture No. 3 consists of:

Complement	(diluted to the proper titer)	1 part
Saline solution		1 part

Mixture No. 1 containing cholesterinized antigen is added to the first tubes of the water-bath set in the amount of 0.4 c.c., and the mixture containing crude alcoholic antigen is added to the first tubes of the ice box set.

Mixture No. 2 containing crude alcoholic extract is added to the second tubes of the ice box set in the amount of 0.5 c.c. The mixture containing cholesterinized antigen is added to the second tubes of the water-bath set.

Mixture No. 3 is added to the third tubes of both sets (water-bath and ice box) in the amount of 0.4 c.c.

The above additions are made with the distributor in the following manner: It is first adjusted to deliver 0.4 c.c., and Mixture No. 3, consisting of complement and saline solution, is added to all third tubes. The distributor does not require washing. Four-tenths c.c. of Mixture No. 1 is then added to the first tubes of the ice box set. The adjustment is changed to deliver 0.5

c.c., and Mixture No. 2 is added to the second tubes of the ice box and water-bath sets, respectively. The distributor is again readjusted to deliver 0.4 c.c., and Mixture No. 1 is added to the first tubes of the water-bath set. Thorough washing of the distributor is now necessary before the final addition consisting of the sensitized cell-amboceptor mixture (0.4 c.c.).

The results of these procedures are shown in Table II.

A few simple calculations enable one to prepare a list showing the amounts of the various ingredients that are required for any given number of reactions.

TABLE II

	TUBE I	TUBE II	TUBE III
	c.c.	c.c.	c.c.
Serum diluted 1/5	0.2	0.1	0.2
Antigen dilution	0.2	0.1	0.0
Complement dilution	0.2	0.2	0.2
Saline solution	0.0	0.2	0.2
Sensitized cell-amboceptor mixture	0.4	0.4	0.4

TABLE III

SHOWING QUANTITIES OF CONSTITUENTS IN MIXTURES NOS. 1, 2, AND 3 THAT ARE REQUIRED FOR FIVE TO ONE HUNDRED TESTS

MIXTURE NO. 1

0.4 C.C. IN FIRST TUBES

Tests	No.	5	10	20	40	80	100
Antigen	c.c.	1	2	4	8	16	20
Complement	c.c.	1	2	4	8	16	20

MIXTURE NO. 2

0.5 C.C. IN SECOND TUBES

Tests	No.	5	10	20	40	80	100
Antigen	c.c.	0.5	1	2	4	8	10
Complement	c.c.	1.0	2	4	8	16	20
Saline solution	c.c.	1.0	2	4	8	16	20

MIXTURE NO. 3

0.4 C.C. IN THIRD TUBES

Tests	No.	5	10	20	40	80	100
Complement	c.c.	2	4	8	16	32	40
Saline solution	c.c.	2	4	8	16	32	40

The following is a brief résumé of the technical operations that have been described above.

1. Prepare a set of tubes corresponding to the number of sera and place 2.0 c.c. of normal saline solution in each tube.

2. Add 0.5 c.c. of corresponding inactivated serum to each tube. Mix thoroughly and leave the pipettes in the tubes.

3. Pipette 0.2 c.c. of the diluted serum into Tubes I and III and 0.1 c.c. into Tubes II.

4. Prepare Mixtures Nos. 1, 2, and 3 for Tubes I, II, and III respectively. (Table III.)

5. Pipette 0.4 c.c. of Mixture No. 3 into all Tubes III.

6. Pipette 0.4 c.c. of Mixture No. 1 (crude alcoholic antigen) into Tubes I of the ice box set.

7. Pipette 0.5 c.c. of Mixture No. 2 (crude alcoholic antigen) into Tubes II of the ice box set.

8. Pipette 0.5 c.c. of Mixture No. 2 (cholesterinized antigen) into Tubes II of water-bath set.

9. Pipette 0.4 c.c. of Mixture No. 1 (cholesterinized antigen) into Tubes I of water-bath set.

10. Incubate the tubes of the cholesterinized antigen set in the water-bath at 38° C. for fifty minutes and those of the crude alcoholic antigen set in the ice box for four hours at from 0° to 8° C.

11. After incubation for four hours, the alcoholic set is removed from the ice box and placed in the thermostat at 38° C. for one-half hour, at the end of which period sensitized cell-amboceptor mixture in the amount of 0.4 c.c. is added to all tubes with the distributor. At the end of the fifty-minute incubation period for the cholesterin set, 0.4 c.c. of the sensitized cell-amboceptor mixture is added in a similar manner.

The number of pipetting operations by this method for each test tube is three: viz., serum, Mixtures Nos. 1, 2, or 3, and sensitized cell-amboceptor mixture. The economy of time is obvious. The maximum time economy, however, results from the use of the automatic distributor. Only three adjustments of the instrument are used; namely, 0.4 c.c., 0.5 c.c., and 0.4 c.c., whereas formerly five adjustments were necessary; viz., 0.1 c.c., 0.12 c.c., 0.16 c.c., 0.2 c.c., and 0.4 c.c. The apparatus requires only one washing from beginning to end, whereas formerly three washings were required.

TECHNIC FOR SPINAL FLUIDS

The time saving feature is equally applicable to spinal fluid Wassermanns. Our routine technic for this consists in testing the fixation capacity of all fluids in three amounts; viz., 0.4 c.c., 0.2 c.c., and 0.1 c.c. These amounts are the equivalents of 2.0 c.c., 1.0 c.c., and 0.5 c.c. with the original Wassermann-Citron technic, and in order to avoid confusion, our reports are translated into terms of the original amounts. In cases that are positive in 0.5 c.c., a further titration is made with smaller amounts (0.08, 0.04, and 0.02).

TABLE IV

SAMPLE SET UP FOR SPINAL FLUID

		TUBE I	TUBE II	TUBE III
Back Row	Spinal fluid	0.4	0.2	0.1
	Antigen dilution	0.0	0.0	0.0
	Saline solution	0.0	0.2	0.3
	Complement dilution	0.2	0.2	0.2
	Sensitized cell-amboceptor mixture	0.4	0.4	0.4
Front Row	Spinal fluid	0.4	0.2	0.1
	Antigen dilution	0.2	0.2	0.2
	Saline solution	0.0	0.0	0.1
	Complement dilution	0.2	0.2	0.2
	Sensitized cell-amboceptor mixture	0.4	0.4	0.4

The "set-up" for each spinal fluid consists of a front and back row of three tubes each. The back-row tubes contain no antigen and are controls, each containing the same amount of spinal fluid as the corresponding front tube.

1. The spinal fluid is pipetted, 0.4, 0.2, and 0.1 c.c., into Tubes I, II, and III respectively of front and back rows.

2. Complement (0.2 c.c.) is pipetted into all back-row tubes.

3. Mixture No. 1 consisting of equal parts of complement and antigen is pipetted into all front-row tubes in the amount of 0.4 c.c.

4. One-tenth c.c. of saline solution is added to Tubes III of the front row.

5. Two-tenths c.c. of saline solution is added to Tubes II of the back row.

6. Three tenths c.c. of saline solution is added to Tubes III of the back row.

7. After incubation 0.4 c.c. of the sensitized cell-amboceptor mixture is added to all tubes of the front and back rows.

SUMMARY

The method described reduces the pipetting operations for the Wassermann test to three for each test tube.

Blood serum is diluted with saline solution as in the Kolmer technic, and the mixture is added to each tube.

Complement antigen, and saline solution are combined in such a manner that all of them are added at one operation by means of a semiautomatic pipetting device.

Sensitized cell-amboceptor mixture is added in a similar manner.

The semiautomatic pipetting apparatus accomplishes a conservation of time.

All ingredients except blood serum are added with the same measuring pipette, thus enhancing precision.

These technical modifications have three manifest advantages: simplicity, rapidity, and precision.

REFERENCES

[1]Jour. Lab. and Clin. Med., 1922, viii, 56.
[2]Am. Jour. Syph., 1921, v, 290.
[3]Jour. Lab. and Clin. Med., 1919, iv.
[4]Am. Jour. Syph., 1922, vi, 680.

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DISCUSSION

Dr. H. R. Brown.—I would like to ask if there were any comparative results of this test with the other tests?

Dr. Groszberg (closing).—Before we used our new method, 416 tests were compared. Twelve divergencies resulted; the new method was more sensitive and gave stronger reactions. In three we had positive reactions where the old method showed negative results, and we found that the patients had chronic syphilis. The comparison was favorable as far as the new method is concerned.

AN ANTIGEN FOR USE IN SERUM TESTS FOR SYPHILIS*

By B. S. Kline, M.D., Cleveland, Ohio

ANTIGEN emulsions, quantitatively prepared from an acetone-insoluble lipoid wax obtained within a few days as described below, give better results in the microscopic slide precipitation test for syphilis than Kahn antigen dilutions and give good results in the Wassermann test.

The antigenic property of acetone-insoluble lipoids in serum tests for syphilis was first reported in December, 1907, by Porges[1] and by Levaditi and Yamanouchi.[2] Since then the work of Nogouchi,[3] Browning, Cruikshank, and M'Kenzie,[4] Kolmer,[5] and others, has established the reliability of acetone-insoluble lipoid solution as an antigen in the Wassermann test. The wax recommended by these workers requires weeks for its isolation. A method of obtaining an acetone-insoluble lipoid wax, thoroughly satisfactory in serum tests for syphilis, within a few days, is described below.

PREPARATION OF THE ANTIGEN

Briefly stated the procedures for the preparation of the antigen are as follows:

Shake a mixture of 200 gm. of beef heart powder, and 1,000 c.c. of absolute ethyl alcohol (99+ per cent) vigorously for from twenty to thirty minutes.

Filter.

Chill the filtrate at 8° to 10° C. for from fifteen to twenty-four hours.

Concentrate the filtrate to 1/20 of the original volume at 45° to 50° C.

Pour into 1,000 c.c. of acetone C. P. *at 35° C.*

Leave at room temperature for thirty minutes.

Decant acetone.

Spread wax in thin layer on filter paper, and allow acetone to evaporate.

Add 20 c.c. of absolute ethyl alcohol (99+ per cent) to each gram of wax.

Shake for several minutes.

Place at 56° C. for thirty minutes; shake for several minutes, then place at 8° to 10° C. for forty-five minutes.

Filter.

Evaporate filtrate to wax (antigen wax) at 45° to 50° C.

Add 10 c.c. of absolute ethyl alcohol (99+ per cent) to each gram of wax.

Shake for several minutes.

Place at 56° C. for thirty minutes; shake for several minutes, then place at 8° to 10° C. for forty-five minutes.

Filter. The filtrate is the antigen.

The details of the preparation of the antigen are as follows:

The two hundred grams of dried heart powder (Difco) is placed in a two liter Erlenmeyer flask.

One liter of absolute ethyl alcohol (99+ per cent) is added.

*From the Laboratory Department, Mount Sinai Hospital.
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After the flask is stoppered with a cork covered with tin foil, it is shaken vigorously by hand or in a shaking machine for from twenty to thirty minutes. (This short extraction removes almost all of the desired antigenic substance in the powder.)

The extract is filtered into a liter cylinder through good grade filter paper of medium texture (Schleicher and Schull No. 597, 32 cm.).

During filtration, the mixture is stirred with a wooden tongue depressor and toward the end, pressed with the cork until the powder is quite dry.

The extract (about 775 c.c.) is placed in the refrigerator at 8° to 10° C. for from fifteen to twenty-four hours.

During this time, a fairly heavy white precipitate settles out. This is filtered off and the filtrate in a large evaporating dish is concentrated about twenty times (to about 35 c.c.) on a water-bath or by an air heater (bathroom heater) at 45° to 50° C. determined by a thermometer bulb within the extract. (If the extract is concentrated much further, the resultant wax is not quite as satisfactory.)

The concentrated extract is poured into 1,000 c.c. of acetone C. P. (Coleman and Bell) at 35° C. in a liter cylinder. (It is important that the acetone be at this temperature. If it is much lower than this, an unsatisfactory sticky wax resembling that of a plain alcoholic extract results. If the acetone is too warm, at 42° C. or more, a considerable amount of the lecithin remains in solution. To raise its temperature, the acetone is placed in an incubator at 37° C. for at least thirty minutes. A liter cylinder is warmed for use at the same time.)

When properly prepared, an abundant, flaky, light brown precipitate quickly forms and settles. That adherent to the sides of the cylinder is dislodged with a long glass rod about fifteen minutes later.

To be sure of complete precipitation, the cylinder is allowed to stand at room temperature an additional fifteen minutes after which the acetone is carefully decanted. The soft brown wax is removed with a glass rod and spread thinly on filter paper of close texture, and placed in a current of warm air (45° to 50° C.) for about thirty minutes, or allowed to stand at room temperature until the odor of acetone is no longer detectable.

The wax is then weighed (about 4 gm. is the average yield) and 20 c.c. of absolute ethyl alcohol (99+ per cent) is added to each gram of wax in a glass-stoppered bottle.

After a few minutes' shaking, the bottle is placed in a paraffin oven (at 56° C.) for thirty minutes to dissolve as much of the wax as possible (the wax is not completely soluble in alcohol).

On removing the bottle from the oven it is shaken for a few minutes and then placed in the refrigerator at 8° to 10° C. for forty-five minutes.

It is then filtered and the filtrate is evaporated down at 45° to 50° C. (water-bath or air heater) resulting in a soft brown wax (antigen wax). The wax is weighed and to each gram, in a glass-stoppered bottle, 10 c.c. of absolute ethyl alcohol (99+ per cent) is added. After the bottle is shaken for a few minutes it is placed at 56° C. for thirty minutes, and then shaken a few minutes. (The wax although obtained from a clear alcoholic solution is again incompletely soluble in alcohol).

The slightly turbid solution is placed at 8° to 10° C. for about an hour, and then filtered. The resultant clear filtrate is the antigen and contains about 8.75 per cent of the alcohol-treated acetone-insoluble wax. (This wax, if repeatedly treated with alcohol or precipitated with acetone, is still incompletely soluble in alcohol.)

The antigen is kept at room temperature.

FORMULAS OF ANTIGEN EMULSIONS FOR THE MICROSCOPIC SLIDE PRECIPITATION TEST

It has been found advisable to use two antigen emulsions in the precipitation test for syphilis: one, very sensitive, giving results in agreement with Wassermann tests done with cholesterinized antigen; the other, less sensitive, giving results in agreement with Wassermann tests done with noncholesterinized antigen.

1. VERY SENSITIVE ANTIGEN EMULSION

0.85 c.c. Distilled water.
1.25 c.c. of 1% Cholesterin (Merck) in absolute ethyl alcohol (99+%). (Prepared in about forty-five minutes by placing in a paraffin oven at 56° C. and shaking a few minutes at fifteen minute intervals.)
0.1 c.c. Antigen.
2.2 c.c. of 0.85% Sodium chloride (C. P. Merck) solution.

2. SENSITIVE ANTIGEN EMULSION

0.85 c.c. Distilled water.
0.75 c.c. of 1% Cholesterin (Merck) in absolute ethyl alcohol (99+%).
0.1 c.c. Antigen.
2.7 c.c. of 0.85% Sodium chloride (C. P. Merck) solution.

These emulsions are prepared as follows:

Into a one-ounce bottle 0.85 c.c. of distilled water is pipetted.

The bottle is held at an angle and the 1 per cent cholesterin (Merck) in absolute ethyl alcohol (99+ per cent) is allowed to run along the side of the bottle.

The bottle is gently rotated from the neck for from fifteen to twenty seconds.

The bottle is held at an angle again and 0.1 c.c. of antigen is pipetted against the side from a 0.2 c.c. pipette (graduated in thousandths).

The bottle is promptly stoppered with a cork covered with tin foil and shaken fairly vigorously (the fluid thrown from bottom to cork and back) for thirty seconds.

Lastly, the 0.85 per cent sodium chloride (C. P. Merck) solution is allowed to run in quite rapidly, the bottle is stoppered again and shaken as previously for thirty seconds.

The emulsions, showing numerous very fine particles at a magnification of 75 times, are now ready for use in the microscopic slide precipitation test for syphilis. They are thoroughly satisfactory immediately after preparation or at any time within three days (kept at room temperature). One drop from a fine capillary pipette equal to about 0.0075 c.c. is the amount added to 0.05 c.c. of serum.

FORMULAS OF ANTIGEN EMULSIONS FOR THE WASSERMANN TEST

1. CHOLESTERINIZED ANTIGEN EMULSION

1 part very sensitive antigen emulsion (see above).
29 parts distilled water.

2. NONCHOLESTERINIZED ANTIGEN EMULSION

0.1 c.c. antigen.
5 c.c. distilled water.

These emulsions are prepared by pipetting the slide test emulsion or antigen first and then adding the distilled water gradually, at the same time shaking the bottle.

DISCUSSION OF ANTIGEN AND FORMULAS

If the alcoholic extract of heart powder is concentrated shortly after its preparation, a wax is obtained that usually contains an impurity which appears as small colorless granular plates in the slide test, and so interferes with the reading. As indicated above, the extract is freed of this impurity by chilling for from fifteen to twenty-four hours.

A precaution absolutely necessary to obtain a satisfactory wax is the avoidance of high temperatures in evaporating the extract. Waxes prepared at temperatures above 60° C. have yielded antigen emulsions giving false as well as true positive results. The antigen wax when properly prepared is a soft brown wax that keeps well in the refrigerator and at room temperature. With it, whenever desired, the antigen is made as detailed above.

In addition to preparing the acetone-insoluble antigen wax from a plain alcoholic extract of heart muscle powder, it has been obtained from an alcoholic extract after preliminary ether extraction (Kahn base) by concentration, acetone precipitation, and alcohol treatment as outlined above. The Kahn base itself, evaporated at 45° C., results in a soft brown wax lighter in color and more greasy than the acetone-insoluble wax. When fanned at room temperature, the Kahn base is found to contain an oily substance in addition to a soft brown wax. It is possible that this oily substance is responsible for the nonspecific granularity that develops in the majority of Kahn antigen dilutions at low temperatures.

A plain alcoholic extract evaporated at 45° C. results in a sticky brown wax which does not emulsify in salt solution. When fanned at room temperature, the plain alcoholic extract results in a sticky brown wax and in abundant transparent oily fat. When this residue is left at room temperature for a number of hours, a white precipitate settles out.

In contrast to the waxes just described, that obtained by fanning (at room temperature) the alcoholic solution of the acetone-insoluble antigen wax, contains no appreciable amount of oily substance, but closely resembles the soft brown wax obtained at 45° C.

The solutions of these waxes likewise differ in character. Plain alcoholic antigens or those following preliminary acetone or ether extraction or those made by adding known substances to an alcoholic extract of previously extracted tissue, prepared a number of times according to a given formula, vary greatly in substances contained and in concentration of desired substances, not a few being unsatisfactory for use. The antigen prepared as described above, however, being of uniform quality and of known concentration, gives best results in serum tests for syphilis, especially in the microscopic slide precipitation test. Whereas the majority of Kahn antigen dilutions are unsatisfactory at low room temperatures because a nonspecific granularity develops, emulsions with the acetone-insoluble antigen remain clear. In addition they are relatively stable and are therefore superior in use to the unstable Kahn antigen dilutions. For these reasons, the former technic of doing the microscopic slide precipitation test for syphilis, in a warm room with ingredients and glassware warm, and within a limited time to insure satisfactory results with the Kahn antigen dilution, has been simplified to the doing of the test with the acetone-insoluble emulsions without any precautions relating to the temperature of the room, glassware and ingredients or to the time (that day) the emulsions are prepared.

In working out the formulas, described above, for the precipitation test, it was found that not only are the quantities of the ingredients important but also the order of their mixture. It is possible to secure uniformly satisfactory emulsions only when the cholesterin is precipitated before the antigen is added.

It was further observed that the sensitivity of the antigen emulsion is dependent more upon the cholesterin content than upon that of the antigen

TABLE I

STABILITY OF EMULSION PREPARED FROM ANTIGEN WAX

DATE	WEEKS AFTER PREPARATION OF EMULSION	AGREEMENT			RELATIVE AGREEMENT			DISAGREEMENT		
		SLIDE TEST (EMULSION) WITH SLIDE TEST (KAHN DILUTION FRESH)	SLIDE TEST (EMULSION) WITH WASSERMANN TEST	SLIDE TEST (KAHN DILUTION FRESH) WITH WASSERMANN TEST	SLIDE TEST (EMULSION) WITH SLIDE TEST (KAHN DILUTION FRESH)	SLIDE TEST (EMULSION) WITH WASSERMANN TEST	SLIDE TEST (KAHN DILUTION FRESH) WITH WASSERMANN TEST	SLIDE TEST (EMULSION) WITH SLIDE TEST (KAHN DILUTION FRESH)	SLIDE TEST (EMULSION) WITH WASSERMANN TEST	SLIDE TEST (KAHN DILUTION FRESH) WITH WASSERMANN TEST
4/ 9/27	4	36	33	33	2	4	4	1	2	2
5/ 6/27	5	18	21	17	4	2	4	1	0	2
5/13/27	6	22	19	19	2	5	5	0	0	0
Total		76	73	69	8	11	13	2	2	4

Evaluation according to the method of Kahn:
Positive reaction + + + +, + + + and + +.
Doubtful Reaction + and ±.
Agreement = Positive or negative by both methods.
Relative Agreement = Positive or negative by one method and doubtful with the other.

TABLE II

SERUM TESTS FOR SYPHILIS WITH CLINICAL COMPARISON

LABORATORY	SLIDE TEST VERY SENSITIVE EMULSION		SLIDE TEST SENSITIVE EMULSION		WASSERMANN TEST*		ANTICOMPLEMENTARY
	Agreement	Disagreement	Agreement	Disagreement	Agreement	Disagreement	
Lakeside Hospital Courtesy of Dr. A. R. Moritz	83	0	82	1	77	5	1
Mount Sinai Hospital	345	4	175	7	335	14	13
Total	428 99.07%	4 0.93%	257 97%	8 3%	412 95.59%	19 4.41%	14 3.15%

*Cleveland method.

Disagreements all in luetic sera: ± to + + + + in one test, negative in another.

itself. Satisfactory emulsions may contain, in a total of 4.4 c.c., from 0.0375 to 0.15 c.c. of the antigen. The cholesterin content, on the other hand, must be more carefully titrated; since the more nearly it approaches the maximum dispersible in a given total, the more sensitive the emulsion becomes. Beyond this point the emulsions are unsatisfactory because of the presence of a nondispersible precipitate.

It was noted that distilled water has a greater dispersing effect on emulsions than has salt solution. On the other hand, emulsions containing salt solution, although losing their antigenic properties sooner, give stronger precipitation reactions than those prepared with distilled water.

Concerning the influence of alcohol on the wax or in the emulsions, it was found that preparations from the concentrated alcoholic solution of the base (antigen) are most satisfactory; since they permit of the dispersion, in a given total, of much more cholesterin than those prepared with the same quantity of the base in from five to ten times the quantity of alcohol. Furthermore they are relatively stable, keeping (at room temperature) their antigenic properties undiminished for three days. Much more stable than these but less satisfactory for routine preparation are emulsions made from the wax itself (no alcoholic dilution). These (at room temperature) retain their antigenic power for weeks (see Table I).

The optimal quantities of the ingredients for the Wassermann antigen emulsions have not yet been determined. The emulsions described above, however, give good results. Titration shows the following:

	HEMOLYTIC UNIT	ANTICOMPLIMENTARY UNIT	ANTIGENIC UNIT
Very Sensitive Emulsion	Greater than fifteen times amount used in the test	Four to six times amount used in the test	One-fourth amount used in the test

The results with the emulsions in the precipitation tests and Wassermann tests are given in Tables II to V.

TABLE III

COMPARISON OF MICROSCOPIC SLIDE PRECIPITATION TESTS AND WASSERMANN TESTS

LABORATORY	SLIDE TEST VERY SENSITIVE ANTIGEN EMULSION AND WASSERMANN TEST (CLEVELAND METHOD)			SLIDE TEST SENSITIVE ANTIGEN EMULSION AND WASSERMANN TEST (CLEVELAND METHOD)			SLIDE TEST VERY SENSITIVE ANTIGEN EMULSION AND SLIDE TEST SENSITIVE ANTIGEN EMULSION		
	Agreement	Relative Agreement	Disagreement	Agreement	Relative Agreement	Disagreement	Agreement	Relative Agreement	Disagreement
Lakeside Hospital Courtesy of Dr. A. R. Moritz.	77	2	4	79	1	3	80	3	0
Mount Sinai Hospital. City Health Department, Courtesy of Dr. H. J. Knapp.	318 422	29 23	15 7	80	12	6	177	19	4
Total	817 91.08%	54 6.02%	26 2.9%	159 87.85%	13 7.18%	9 4.97%	257 90.81%	22 7.78%	4 1.41%
	871 97.1%		26 2.9%	172 95.03%		9 4.97%	279 98.59%		4 1.41%

TABLE IV

SERUM TESTS FOR SYPHILIS WITH CLINICAL COMPARISON

DATE	SLIDE TEST VERY SENSITIVE ANTIGEN EMULSION		SLIDE TEST KAHN ANTIGEN DILUTION		SLIDE TEST SENSITIVE ANTIGEN EMULSION		WASSERMANN ETH. INSOL. NONCHOL.		WASSERMANN ACETONE INSOL. CHOL.		WASSERMANN ACETONE INSOL. NONCHOL.		ANTI-COMPLEMENTARY
	Agreement	Disagreement	Agreement	Disagreement	Agreement	Disagreement	Agreement	Disagreement	Agreement	Disagreement	Agreement	Disagreement	
4/29/27 to 5/13/27							137	6					
7/22/27 to 8/ 2/27	225	4	225	4									
8/ 5/27 to 8/16/27	120	0			175	7	198	8	195	11	161	12	13
TOTAL	345 98.85%	4 1.15%	225 98.25%	4 1.75%	175 96.15%	7 3.85%	335 95.99%	14 4.01%	195 94.66%	11 5.34%	161 93.06%	12 6.94%	13

Disagreements: All in luetic sera, ± to ++++ in one test, negative in another.

Disagreements. All in luetic sera, ± to + + + + in one test, negative in another.

TABLE V

COMPARISON OF SERUM TESTS FOR SYPHILIS

DATE	SLIDE TEST VERY SENSITIVE ANTIGEN EMULSION AND WASSERMANN TEST (ETH. INSOL. NONCHOL. ANTIGEN)			SLIDE TEST KAHN ANTIGEN DILUTION AND WASSERMANN TEST (ETH. INSOL. NONCHOL. ANTIGEN)			SLIDE TEST VERY SENSITIVE ANTIGEN EMULSION AND WASSERMANN TEST (ACET. INSOL. CHOL.)			SLIDE TEST SENSITIVE ANTIGEN EMULSION AND WASSERMANN TEST (ACET. INSOL. NONCHOL.)			SLIDE TEST VERY SENSITIVE ANTIGEN EMULSION AND SLIDE TEST KAHN ANTIGEN DILUTION			SLIDE TEST VERY SENSITIVE ANTIGEN EMULSION AND SLIDE TEST SENSITIVE ANTIGEN EMULSION		
	Agreement	Relative agreement	Disagreement	Agreement	Relative agreement	Disagreement	Agreement	Relative agreement	Disagreement	Agreement	Relative agreement	Disagreement	Agreement	Relative agreement	Disagreement	Agreement	Relative agreement	Disagreement
4/29/27 to 5/13/27 7/22/27 to 8/ 2/27	190	22	11	190	21	12												
8/ 5/27 to 8/16/27	128	7	4				159	25	7	80	12	6	210	19	2	177	19	4
TOTAL	318 87.84%	29 8.01%	15 4.15%	190 85.20%	21 9.42%	12 5.38%	159 83.25%	25 13.09%	7 3.66%	80 81.63%	12 12.25%	6 6.12%	210 90.91%	19 8.22%	2 .87%	177 88.50%	19 9.5%	4 2%
	347 95.85%		15 4.15%	211 94.62%		12 5.38%	184 96.34%		7 3.66%	92 93.88%		6 6.12%	229 99.13%		2 .87%	196 98%		4 2%

SUMMARY

1. Antigen emulsions, quantitatively prepared from an acetone-insoluble lipoid base, obtained within a few days as described above, give better results in the microscopic slide precipitation test for syphilis than Kahn antigen dilutions and give good results in the Wassermann test.

2. Formulas giving the optimal quantities of the ingredients for the precipitating emulsions are detailed above. The order of mixture in preparing these emulsions is likewise important. It is possible to secure uniformly satisfactory emulsions only when the cholesterin is precipitated before the antigen is added.

3. The emulsions for the microscopic slide precipitation test prepared as described above have replaced Kahn antigen dilutions in this laboratory for the following reasons:

Antigen Emulsions	*Kahn Antigen Dilutions*
(a) Relatively stable, satisfactory for at least three days.	(a) Unstable, satisfactory for an hour or an hour and a half at most, usually less.
(b) May be used immediately after preparation.	(b) Must stand from ten to fifteen minutes before use.
(c) Satisfactory at low room temperatures as well as at ordinary ones.	(c) Majority unsatisfactory at low room temperatures.

(d) The very sensitive emulsion is more sensitive than the Kahn antigen dilution and no less specific.

(e) The emulsions are more uniform in quality and quantity than the Kahn antigen dilutions.

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DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

CLINICAL AND EXPERIMENTAL

TABES DORSALIS: Atypical Tabes Dorsalis (Forme Fruste), Bennett, A. E. Am. Jour. Med. Sc., October, 1925, clxx, 538.

Atypical or "forme fruste" type of tabes is the most frequently missed form of the disease. Gastric crises in this variety often lead to unnecessary abdominal operations.

Incomplete histories, careless examinations and infrequent spinal punctures in doubtful cases account for most errors. Gastric crises occur in from 10 to 20 per cent of all cases of tabes dorsalis.

Reliable reports indicate that more than 10 per cent of the tabetics have needless operations performed at least once upon a mistaken diagnosis. There are frequent reports in the literature of patients having had four or five abdominal operations when the cause of the symptoms was nerve root pains of syphilis.

The classical signs of tabes are absent in approximately one-half of the early cases, when abdominal pains may be present. The knee jerks are normal or exaggerated in at least 25 per cent of early cases. The same applies to ataxia, Argyll-Robertson phenomenon and other later signs. Approximately 10 per cent of cases remain atypical throughout the entire course of the disease.

A complete life story of the individual with a careful analysis of the onset and development, a careful neurologic examination and a spinal fluid study should be made where there is the slightest doubt as to a diagnosis.

In patients presenting recurrent abdominal attacks with no relief from previous operations, the possibility of an atypical form of tabes dorsalis should be very firmly impressed upon the minds of surgeons, in order to decrease the all too frequent incidence of unnecessary operations upon tabetics.

TOXEMIAS OF PREGNANCY: Lactic Acid in the Toxemias of Pregnancy, Stander, H. J. and Radelet, A. H. Bull. Johns Hopkins Hosp., August, 1926, xxxix, No. 2, p. 91.

In pregnancy complicated with low reserve kidney, the lactic acid in the blood is increased. This accumulation of lactic acid disappears as the patient improves.

In pregnancy complicated with nephritis, there is a similar accumulation of lactic acid in the blood, which disappears with the improvement of the patient.

The factors which may possibly play a rôle in the accumulation of lactic acid in these two types of pregnancy toxemia are:

a. Decreased elimination of lactic acid.
b. Decreased oxidation and interference with the resynthesis of lactic acid into glycogen.
c. A possible disturbance of the hydrogen-ion concentration of the blood.

HODGKIN'S DISEASE: Unique Features of Hodgkin's Disease (Lymphogranulosis), Barron, M. Arch. Path. and Lab. Med., November, 1926, ii, No. 5, p. 659.

From a study of 27 cases (including 23 autopsies) the following conclusions are reached:

Hodgkin's disease is twice as common among males as among females. Fifty per cent of the cases occur during the second and third decades of life, although cases can occur in practically all ages. The average duration of this disease is under two years.

An associated infection with tuberculosis is not infrequent. The retroperitoneal and prevertebral lymph nodes are frequently involved, in our series exceeding that of the cervical nodes. The involvement of the spleen is a characteristic feature of this disease, and, contrary to the general opinion, this enlargement may at times be enormous. Involvement of the vertebrae may lead to a diagnosis of Pott's disease. No tissue or organ of the body is exempt from involvement.

The characteristic histologic picture is identical for every organ and tissue of the body. The Dorothy Reed cells or Sternberg's giant cells are characteristic of the disease.

The blood picture is not characteristic. Some cases, however, present marked eosinophilic leucocytoses, and this when associated with an enlarged spleen or enlarged lymph nodes is helpful in suggesting a correct diagnosis. A severe anemia of the secondary type is present in practically all cases in the terminal stages of the disease.

Some cases of Hodgkin's disease present characteristic types of chronic relapsing fever which probably constitute an integral part of the syndrome and are not due to secondary infections. A case of Hodgkin's disease with Pel-Ebstein type of fever is here reported which is unique in the regularity of the recurrences of the febrile attacks.

Pruritus is a common cutaneous manifestation in Hodgkin's disease, occurring in from 10 to 20 per cent of the cases. It may appear early, at times as an initial symptom, and may precede by months or even years the appearance of any visible skin lesions, when such lesions occur.

Hodgkin's disease (lymphogranulomatosis) is an entity anatomically as well as clinically. It is an infectious granuloma caused by an organism not yet identified.

Reasons are here presented to suggest that the etiologic agent is probably an animal parasite.

Neither tuberculosis nor the tubercle bacillus bears any etiologic relationship to this disease.

Caseation necrosis may be extensive in the lymph nodes of Hodgkin's disease and may thus resemble the necrosis of tuberculosis and lead to a mistaken diagnosis.

The pseudodiphtheria bacillus—B. hodgkini—has no casual relationship to this disease.

The prognosis of this disease is always hopeless. No chronic infectious disease caused by any known bacterium is so invariably fatal.

Roentgen-ray therapy may cause temporary symptomatic relief, but apparently has no effect on the progress of the disease.

An incontrovertible diagnosis can be established only through a biopsy.

NEUROSYPHILIS AND MALARIA: Treatment of Neurosyphilis by Malaria, O'Leary, P. A., Goeckerman, W. H., and Parker, S. T. Arch. Dermat. and Syph., November, 1926, xiv, No. 5, p. 550.

A report of the second year's observations on thirty-five patients treated by malarial infection and on an additional sixty-five patients treated during the past year.

The continued observations on the thirty-five patients, treated by the fever therapy of Wagner von Jauregg and followed for a period of two years, has convinced us of the value of the method in the treatment of parenchymatous neurosyphilis, particularly of the general paralytic type. It also suggests that some of the complications of neurosyphilis, such as gastric crisis, persistent lightning pains and optic atrophy, will be ameliorated by fever therapy. There has been a mortality of 8 per cent in the 100 patients treated, of which five deaths (5 per cent) may be attributed, directly or indirectly, to the malaria.

Although there has been a slight increase in the number of remissions produced during the second as compared with the first year, the outstanding feature of the study has been that thus far none of the patients who were improved has shown any evidence of relapse, but all have continued to improve. Also, there has been a reduction in the degree of positiveness of the serologic tests of the blood and spinal fluid in the group as a whole. The outstanding serologic observation has been the absence of serologic relapse in any of the patients reexamined. The authors' experience has taught them to favor the use of the present-day antisyphilitic remedies three or four months after the course of malaria, based

on the observation that those patients who received intraspinal treatment, for example, after the course of fever, have shown better clinical and serologic results than those who did not.

They believe that some of the therapists who have been disappointed in the fever therapy of Wagner von Jauregg are discouraged because the signs and symptoms of the advanced phases of the disease and the result of actual destruction of tissue have not been obliterated. The authors have not anticipated such phenomena but have been encouraged by obtaining definite symptomatic benefit. In their previous communication, it was pointed out that the patients with general paralysis were in the early phases of the disease, and not far enough advanced to warrant institutional care.

The authors are now more enthusiastic about the results of the treatment with malaria than they were last year. Their past experience, however, with the treatment of neurosyphilis, particularly early general paralysis, prevents them from making conclusive deductions at this time of the value of the method. A more extended trial with frequent observations is warranted by the results thus far obtained.

WASSERMANN REACTION: The Occurrence of the Wassermann Reaction After Milk Injections, Kirschenblatt, D. and Narinjan, A. Deutsch. med. Wchnschr., July, 1926, lii, 1298.

This investigation was stimulated by the article of Felix Klopstock in the Deutsch. med. Wchnschr., 1925, No. 41. In a number of patients with different acute and chronic affections after excluding syphilities and strict control by the Wassermann reaction an attempt was made to find a reaction corresponding to the syphilitic blood change by means of several milk injections. Fifteen patients, among them four women were given preliminary treatment with boiled cow's milk every three to four days in doses of 2, 5, 8 and 10 c.c.

With two exceptions all the patients showed general reactions and fever, often high (up to 39°C.); in the arthritics and rheumatics there were also focal reactions. And yet there was not a Wassermann reaction in a single case, the blood being examined both in the febrile stage and in the resting interval between the injections eighteen to thirty hours after each milk injection. The Wassermann reaction was performed with two antigens, a specific one and a normal one (oxheart by Bordet-Roulens method). These attempts to bring about a positive Wassermann reaction, even temporarily in diseased individuals by the parenteral injection of milk were negative.

SICKLE CELL ANEMIA: The Sickle Cell Phenomenon, Cooley, T. B. and Lee, P. Am. Jour. Dis. Child., September, 1926, xxxii, 334.

Of 400 colored patients (children) 30 or 7.5 per cent were found to have sickle cells. Two new facts are added: (1) In preparations kept at incubator temperature, the sickle cells disappear rapidly, leaving the round cells behind; (2) The cells of one of the patients having a hemolytic anemia are rapidly hemolyzed at incubator temperature in their own serums and in serums from normal bloods, while the serum of the patient was not hemolytic for normal cells.

It is suggested that these findings imply a special vulnerability of the sickle cell.

The authors consider the sickle cell to be a congenital, familial and hereditary phenomenon possibly transmitted chiefly through the mother.

They believe, also, that the presence of sickle cells in the blood does not in itself imply any essential anemia, active or latent. Most of the patients with sickle cells are as normal in all other ways as the rest of the colored population in Detroit. The term "sickle cell anemia" should be reserved for patients with definite hemolytic anemia and another term used for the condition without symptoms. "Sicklemia" is suggested.

They believe that sickle cell anemia is a true disease entity to be considered as another form of familial hemolytic jaundice and that there may be a hemolytic "diathesis" due probably to a special vulnerability of the sickle cells to some hemolytic agent.

They speculate as to the possible sequelae dependent upon a consideration of "sicklemia" as a dominant mendelian characteristic.

CATARRHAL JAUNDICE: The Pathology of Icterus Catarrhalis, Klemperer, P., Killian, J. A., and Heyd, C. G. Arch. Path. and Lab. Med., November, 1926, ii, No. 5, p. 631.

The so-called "icterus catarrhalis" is neither a morbid nor a pathologic entity. Three forms can be differentiated:

1. Icterus due to obstruction of the common duct following gastrointestinal catarrh—true catarrhal jaundice.

2. Icterus due to degeneration and multiple necrosis of the liver, hematogenous in origin.

3. Icterus due to cholangitis, mostly of hematogenous origin.

The etiologic factor of group 2 is not known; bacterial toxins of various types have to be considered. It is probable that in group 3 atypic strains of B. paratyphosus are of etiologic importance. It is possible that groups 2 and 3 frequently merge with each other.

The evidence of hepatic derangement in cases of group 2 suggests careful observation of these cases and dietary regulations in order to prevent further damage to the liver. The history, the presence of urobilin in the urine and the positive results of practically all the liver function tests permit a differentiation from group 1. Further studies are necessary, however, in order to make a correct differentiation of groups 2 and 3 possible.

AUTOHEMOTHERAPY: Effect of Autohemotherapy on the Blood Picture, Hirsch, L. Deutsch. med. Wchnschr., July, 1926, lii, 1302.

The author made a systematic study of autohemotherapy on the blood picture, giving 5 c.c. of the patient's own blood intramuscularly or subcutaneously or the same amount of the serum intramuscularly. There were cases of angina, grippe, bronchitis, asthma, pneumonia, pleurisy, chronic and acute polyarthritis. Blood smears were made and blood taken for leucocyte counts shortly before the injection and five, ten and fifteen minutes and three, six and nine hours afterward. No change in the blood picture due to digestion was seen in the smears taken two hours after meals. More importance was attached to the relative than to the absolute leucocyte counts, for often there is no parallelism between the absolute and relative counts.

In all 31 tests were made. In 12 of these there was no effect on the blood picture, 6 of these being after intramuscular injection, 5 after subcutaneous and 1 after injection of serum. Ten of these 12 were in the subjects with normal blood pictures; it is natural that bone marrow that is not affected by the disease should not react to the patient's own blood. Yet a minority of the patients with normal blood reacted to the injections. Blood pictures shifted to the left were affected in over 80 per cent of the cases, and in the majority both the lymphatic and myeloid part was affected. But in general there was less reaction of the lymphocytes than of the neutrophils. Among the 19 cases that reacted 13 showed an improvement in the blood picture (increase in lymphocytes, decrease in rod-nucleated forms), in 6 it grew worse (increase of rod-nucleated forms and decrease of lymphocytes). In the improved blood pictures there was generally an increase in the eosinophiles up to 8 per cent of the total leucocytes. No rule could be determined in the variations in the monocytes. Only changes of at least 25 per cent as compared with the beginning values were considered. Frequently the changes, particularly in the rod-nucleated forms were 50 per cent or more.

The cause of the effect on the blood picture is probably a stimulant action of the injected blood or products of its catabolism on the bone marrow or on the distribution of the blood in the different organs. If it is a question of change only in the absolute counts it can be explained by changes in distribution. But where the proportion of the different kinds of cells is changed the bone marrow must be involved. The stimulus on the function of the bone marrow is probably brought about through the intermediation of the vegetative nervous system.

In the patients in whom the blood picture did not change or grew worse the clinical picture did not change noticeably in either direction. But even in the patients with an improved blood picture the clinical picture was improved in only 50 per cent. But the experiments were at first made with doses that are far less than those used therapeutically.

LABORATORY TECHNIC

FEVER AND SYPHILIS: The Therapeutic Effect of Fever in Experimental Rabbit Syphilis, Schamberg, J. F., and Rule, A. M. Arch. Dermat. and Syph., September, 1926, xiv, No. 3, p. 243.

It is possible to prevent syphilitic infection in rabbits after testicular implantation of the specific virus if the rabbits are given a series of baths at a temperature of 45° C. (113° F.) within three to four days after inoculation. An average rise of temperature of 4° F. is induced.

How many baths are necessary to sterilize the infection is not yet definitely known, but at the date of this communication it would appear that about nine are necessary.

Infection can also be prevented by heating the spirochetic suspension on a water-bath at 40° C. (104° F.) for one hour before inoculation.

It would appear that the thermal death point outside the body of Spirocheta pallida is about 41° C. (105.8° F.) with an exposure of approximately six hours.

While these experiments indicate that Spirocheta pallida in the rabbit cannot withstand high fever, it is not definitely proved that this applies to human syphilis.

Whether these experiments shed any illumination on the mode of action of malaria in syphilis can only be determined by further investigation.

CARCINOMA: Grading the Malignancy of Carcinoma and Its Practical Application, Broders, A. C. Arch. Path. and Lab. Med., September, 1926, ii, 376.

Broders uses the following classification:

If an epithelioma shows a marked tendency to differentiate, that is, if about three-fourths of its structure is differentiated epithelium and one-fourth undifferentiated, it is graded 1; if the differentiated and undifferentiated epithelium are about equal, it is graded 2; if the undifferentiated epithelium forms about three-fourths and the differentiated about one-fourth of the growth, it is graded 3; if there is no tendency of the cells to differentiate, it is graded 4. Of course, the number of mitotic figures and the number of cells with single large deeply staining nucleoli (one-eyed cells) play an important part in the grading.

He further modified this conception as follows:

Instead of a Grade 1 epithelioma in which about three-fourths of the cells are differentiated and one-fourth undifferentiated, should be substituted a Grade 1 epithelioma, one in which differentiation or self-control ranges from almost 100 to 75 per cent, and undifferentiation from almost nothing to 25 per cent; a Grade 2 epithelioma, one in which differentiation or self-control ranges from 75 to 50 per cent, and undifferentiation from 25 to 50 per cent; a Grade 3 epithelioma one in which differentiation or self-control ranges from 50 to 25 per cent, and undifferentiation from 50 to 75 per cent, and a Grade 4 epithelioma, one in which differentiation or self-control ranges from 25 per cent to practically nothing, and undifferentiation from 75 to practically 100 per cent. So far as an estimation of the ultimate result is concerned, this revision will have no effect on Grades 1 and 2, but will affect slightly Grades 3 and 4, because a small percentage of neoplasms, formerly classified Grade 3, will now be classified Grade 4. In other words, the most malignant of the Grade 3 neoplasms will be classified in Grade 4.

He calls attention to the practical application of this procedure in these words:

"Turning to the practical side of the grading of cancer, it is well known that cancer of Grade 1 shows practically no tendency to metastasize, and therefore in dealing with such neoplasms it does not seem necessary to remove the regional lymph nodes. This saves the patient unnecessary operative procedures. As practically all cancers of Grade 4 with metastasis prove fatal sooner or later, the patients should not be subjected to an operation involving the regional lymph nodes unless they are in close proximity to the primary growth, cancer of the stomach for example. Judd, New, and Figi believe it is useless to perform block dissection of the neck in the presence of a Grade 4 epithelioma of the

lip, tongue, cheeks, floor of the mouth or antrum, etc. In cases of cancer of Grade 2 and in a certain proportion of those of Grade 3, with metastasis, removal of the regional lymph nodes offers a permanent cure in a fair number of cases, as evidenced by the fact that ten (33.3 per cent) of thirty patients with squamous cell epithelioma of the lip of Grades 2 and 3, with metastasis in one group of submaxillary lymph nodes, were living and well on an average of six and one-fifth years after removal of the nodes.''

TUBERCULOSIS AND ASPERGILLOSIS: Aspergillosis of the Lungs and Its Association with Tuberculosis, Lapham, M. E. Jour. Am. Med. Assn., September 25, 1926, lxxxvii, 1032.

Lapham summarizes from the literature the reports of pulmonary aspergillosis in human beings; calls attention to the difficulty of differentiating this condition from tuberculosis and expresses the belief that it is a more frequent complication or concomitant of tuberculosis than is recognized. She argues for routine cultures of the sputum in tuberculosis and says:

''Here is a disease that is capable of causing pleurisy, acute and chronic; bronchitis, acute and chronic; pneumonias, acute and chronic; emphysema; bronchiectasis; atelectasis; sclerotic fibrosis; tubercles; cavities; endarteritis; thrombosis; infarcts; hemorrhages; asthma. Would it be strange if such a disease should seriously complicate or even inhibit recovery in a case of tuberculosis?''

She concludes that we are thoroughly ignorant of the frequency of aspergillosis both as a primary and as a secondary disease.

We have no idea how much aspergillosis of the lungs predisposes to tuberculosis in human beings or in cows.

We do not know how much it impedes or even inhibits recovery in cases of tuberculosis.

We have no idea whatever as to its association with acute respiratory diseases.

We know that it affects cows much as it does human beings.

We know that it gives the same reaction to tuberculin that tuberculosis does.

Should we apply this knowledge to the study of the tuberculosis of dairy herds?

In order to gain adequate information as to the frequency and importance of this disease, should not a systematic research study be made:

1. By determining the percentage of aspergillosis cases among the tuberculosis cases in the large tuberculosis sanatoriums.

2. By determining the percentage of cases of aspergillosis in the lungs at necropsies.

3. By determining the percentage of cases of aspergillosis in cases of respiratory diseases.

4. By applying these principles to dairy herds.

MEASLES TOXIN: Its Preparation and Application as a Skin Test, as an Immunizing Agent, and for the Production of an Antitoxin, Ferry, N. S. and Fisher, L. W. Jour. Am. Med. Assn., March 27, 1926, lxxxvi, 932.

The organism investigated was isolated in pure culture from the blood of patients in the early stages of the disease. It is a Gram-positive, aerobic, green-producing streptococcus appearing both in pairs and in chains. It differs from the organism described by Tunnicliff in growing luxuriantly in the presence of oxygen and in producing a soluble toxin specific for measles.

Isolation was accomplished by planting 10 c.c. of venous blood in 0.25 liter flasks of Hibler medium and, after incubation for twenty-four hours, plating directly on sheep blood agar. After twenty-four-hour incubation the plates were planted to blood agar slants or 0.2 per cent broth.

The organism has been named tentatively Streptococcus morbili.

The Berkefeld W filtrate from a six day growth in 0.2 per cent glucose broth contained a soluble toxin.

Of 35 persons with no history of measles, 14 gave a positive skin reaction to the filtrate when injected intradermally in 0.1 c.c.

Of 30 children with a positive measles history none reacted positively; two adults of 30 with positive histories reacted positively.

Positive reactions were obtained in the preeruptive and early stages of measles, while negative reactions occurred in the later and convalescent stages.

When mixed with convalescent serum no reactions were obtained.

When mixed with artifically prepared antitoxin no reactions were obtained in susceptible individuals.

When mixed with scarlet fever antitoxin the measles toxin was not neutralized.

Persons giving positive reactions are made to give negative reactions by the injection of measles convalescent serum.

Serum from measles cases agglutinates this streptococcus.

Intracutaneous injection into rabbits produced a rash suggestive of measles.

Animals injected with measles toxin produce serum with antitoxic properties.

CANCER SERUM: The Reducing Power of Cancer Serum, Mondain, C., Dauris, R., and Beck, J. Compt. rend. Soc., de biol., April 23, 1926, xciv, 963.

Thomas and Binetti having suggested that cancer serum might be distinguished from normal serum by the rapidity with which it reduced methylene blue when mixed with an extract of cancer tissue, the authors studied the mechanism of this reaction and conclude that fresh cancer serum in the absence of neoplastic extract is incapable of decolorizing methylene blue. Thomas and Binetti's extract decolorized methylene blue by itself. The mechanism of the reaction proposed by Thomas and Binetti for the serum diagnosis of cancer is not therefore due to a high degree of reducing power in the cancer serum. It is due simply to the reducing action of bacteria and their secretions, the bacteria necessary to the production of the reaction being found in the extract which is used for the reaction.

MENINGITIS: A Case of Sporotrichosis Meningitis, Hyslop, G. H., Neal, J. B., Kraus, W. M., and Hillman, O. Am. Jour. Med. Sc., November, 1926, clxxii, No. 5, p. 726.

Sporotrichosis infection of the nervous system has not been observed previously.

The patient was a girl fifteen years old, who became ill December 30, 1924, and died June 19, 1925. The infection involved the posterior fossa largely, had a febrile onset, and ran a chronic remitting course. There were slight exacerbations about every ten days, accompanied by a slight fever.

Interesting clinical occurrences were tetanoid attacks, microptic hallucinations, and several temporosphenoidal seizures.

The diagnosis was made by the presence of sporotrichia spores and mycelial forms in the spinal fluid.

Iodid therapy was of no avail. Experiments with radium intravenously gave interesting results.

Culture and animal inoculation results were negative.

Microphotographs of the spores seen in the spinal fluid and of the tissue from the patient's brain are exhibited.

Autopsy of the patient showed a chronic diffuse leptomeningitis most marked in the posterior fossa, a moderate edema of the brain and the presence of a peculiar gelatinous exudate here and there beneath the pia. Microscopically, there was shown a chronic productive leptomeningitis but no inflammatory involvement of the brain substance.

REVIEWS

Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.

*Outlines of Common Skin Diseases**

THIS little book is intended to serve as a simple and concise guide to the diagnosis of the more common diseases of the skin. It is in no sense of the word a textbook, nor is it intended to be, but rather an outline suggesting a systematic course of procedure from which the student or practitioner may go to larger texts for detailed information.

A short classification, embracing the common skin diseases only, is followed by a very practical outline of the method of making an examination and this by a table of regional distribution. The common skin diseases are then outlined, classed in accordance with their primary lesions.

The little book contains a large amount of information and is, in effect, a set of student's notes.

Scarlatina is listed as among the diseases of unknown etiology in spite of the rather general acceptance of the work of the Dicks.

There are a few typographic errors, such as "a women" for "a woman" on page 48.

In the endeavor to be concise the style has become somewhat jerky and almost too condensed, and occasional abbreviations, such as "mercurial stomat." seem unnecessary.

It is unusual to see so small a book so profusely illustrated.

Basal Metabolism in Health and Disease†

WHILE the subject of basal metabolism has become of considerable importance to the practitioner of medicine within recent years, like all subjects which are in the course of development, much of the literature has been written primarily for research workers and physiologists. In this book Dr. DuBois, whose relation to the development of basal metabolic studies needs no comment, has brought basal metabolism into the domain of clinical medicine and has presented, for the student and the practitioner, a comprehensive, clearly written, and eminently practical review of this important subject.

The volume, a second edition, has been thoroughly revised throughout.

Beginning with a brief but thorough historical review, the metabolism of carbohydrates, fats, and proteins is next discussed in an interesting and understandable manner, the physical laws related to the calculation and understanding of metabolic rates are next covered, after which the gases of the body and their relation to internal and external respiration are thoroughly discussed.

Following is an explanation and review of the general principles of respiration apparatus, and the methods of calculation involved in their use concerning which Dr. DuBois emphasizes that "the man who conducts metabolism experiments should be perfectly frank with himself regarding the accuracy of his work. He should estimate the possible percentage of error and never try to conceal from himself the fact that the technic of a certain experiment was rather poor."

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*Outlines of Common Skin Diseases. By T. C. Gilchrist, Clinical Professor of Dermatology, Johns Hopkins University. Cloth. Pp. 54, with 53 illustrations. Williams & Wilkins Co., Baltimore.

†Basal Metabolism in Health and Disease. By E. F. DuBois, M.D., Associate Professor of Medicine, Cornell. Second Edition. Cloth. Pp. 431, with 92 illustrations. Lea and Febiger, Philadelphia, 1927.

NOTE: In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

After a review of the methods and underlying the estimation of the body surface area, the factors which may influence the normal basal metabolism are discussed in chapter VII, and the following chapter covers a consideration of the selection of normal standards. These chapters are extremely valuable as clarifying the interpretation of basal metabolic readings and should be read by every practitioner interested in the subject or attempting to utilize the application of this method of examination to the study of disease.

After a brief review of the theories of basal metabolism the remainder of the text (Part II—208 pages) is devoted to the significance of basal metabolic determinations in disease.

The relation of undernutrition, overnutrition, and obesity to basal metabolism is reviewed in an interesting and practical manner following which 46 pages are devoted to the basal metabolism in diabetes and 56 pages to its relation to thyroid diseases.

The remainder of the book is given to a discussion of basal metabolism and the adrenal, pituitary, and sex glands; basal metabolism in diseases of the blood, in diseases of the heart and kidneys, and in fever; water metabolism, and the influences of diseases of the nervous system on the basal metabolism.

This is a book which few can afford to be without.

*The Chemical Aspects of Immunity**

A COMPREHENSIVE monograph by the author of *Chemical Pathology* of exceeding interest to chemists and immunologists.

Manual of Operative Surgery†

THE sixth edition, thoroughly revised, of a well-known handbook of operative surgery, clearly written, excellently illustrated, and comprehensive in scope, though compact in form.

The Heart‡

IN THIS little volume the author has endeavored to summarize in a brief but clear fashion the main principles of cardiology.

It is intended for students and for practitioners who desire to review rapidly the modern views of the cardiovascular system.

Its chief value should be as an impetus leading to more extended and comprehensive studies of this important and complicated subject.

Bacteriology for Nurses§

ANYONE who has attempted to teach bacteriology to nurses will appreciate the difficulties involved, especially in view of the short time allowed by the curriculum for the presentation of this and allied subjects. It is very debatable whether or not many subjects now included in the nursing curriculum and especially the extent to which they are supposed to be covered, are really necessary for the training of a nurse, and it is relatively certain that a satisfactory working knowledge of them is more often the exception than the rule.

Certainly, however, a knowledge of the relation of bacteria to the production of disease and some conception of the mechanism involved in the prevention of disease is essential

*The Chemical Aspects of Immunity. By H. Gideon Wells, Professor of Pathology, University of Chicago. Cloth. Pp. 254. The Chemical Catalog Co., New York, 1925.

†Manual of Operative Surgery. By Sir H. J. Waring, Examiner in Surgery, University of Oxford. Cloth. Pp. 868, with 618 illustrations. Price $5.25. Oxford University Press, 1927.

‡The Heart. By A. G. Gibson, Physician to the Radcliffe Infirmary. Cloth. Pp. 108, with 15 illustrations. Oxford University Press, 1926.

§Bacteriology for Nurses. By H. Fox, M.D., Director William Pepper Laboratory, University of Pennsylvania. Fourth edition. Cloth. Pp. 242, with 67 engravings, and 7 colored plates. Lea and Febiger, Philadelphia, 1926.

for the intelligent care of the sick. To supply such information is the purpose of Dr. Fox's little book.

Textbooks of bacteriology for the use of nurses are likely to fall into two errors: they are either too comprehensive and technical to come within the field or the comprehension of the nurse, or so elementary as to remind one of the bed-time story.

Dr. Fox has endeavored, and with some success, to find a middle road. The volume is attractively printed and very well illustrated and has been revised to include the more recent advances in this subject.

*Principles of Physical Chemistry**

AS IS stated in the introduction to this book: "Even the most exalted members of the human family play their parts against a background of semipermeable membranes separating watery solutions of salts, proteins, and other substances," so that, " it is necessary, therefore, that those who some day may be called upon to diagnose and to heal their ills should know something of the complex of chemical events which make up life."

The usual textbook of physical chemistry is too large and too abstract for the needs of the medical student, and the author has endeavored, therefore, to compress into a small volume, a strictly utilitarian text which covers the essentials necessary for an understanding of a complex subject applicable to its relation to the study and practice of medicine.

The book is divided into two main parts: (1) theoretical and (2) practical, embodying a series of laboratory exercises.

The volume is quite practical, well and clearly written, and should be of great use to the student and as a source of reference and review to the practicing physician.

Allergic Diseases†

IN THIS small volume the author records the results of his investigations of allergic diseases as encountered by him in Holland since 1919.

In view of the fact that he found sensitization to Walker's allergens to be relatively infrequent, he developed certain "non-specific" methods of treatment which are given in detail and from the use of which 50 per cent of cures were obtained in asthma; 30 per cent of cases being improved, and 20 per cent uninfluenced, somewhat better results being obtained in hay fever and migraine.

As a result of his studies in Holland and Switzerland Van Leeuwen is convinced that in other low regions as well, influences of climate are the main causes of asthmatic and allergic attacks, these climatic influences being identified with a particular sort of allergens (climate-allergens or miasms) occurring in the air in lower countries and absent or present only in minor degree in higher altitudes.

These climate-allergens are, for the most part, decomposition products of various materials, or products of the metabolism of various animal or vegetable parasites, the occurrence of which is due to the influence of climate, temperature, humidity of the air, etc.

The book is, in essence, the story of the investigations upon which these etiologic concepts are based and a recital of the various methods of treatment which the author has developed, among them the use of tuberculin and, particularly, the isolation of patients in a "miasm-free" chamber.

The author believes that an important factor in the etiology of allergic conditions is an increased permeability or vulnerability of the skin and mucus membranes and that allergic diseases are caused by the inhalation or ingestion of toxic substances through such vulnerable spots, the two important factors being increased permeability and deficient immunization.

The final seventy-two pages of the book are devoted to a detailed account of the author's methods of treatment, including methods directed toward the production of anti-

*Principles of Physical Chemistry for Medical Students. By P. M. T. Kerridge. Cloth. Pp. 134, with 22 illustrations. Oxford University Press, 1927.

†Allergic Diseases. By W. Storm Van Leeuwen, M.D., Director, Pharmaco-Therapeutic Institute, Leyden, Holland. Cloth. Pp. 142, with 3 illustrations. J. B. Lippincott Co., Philadelphia.

anaphylaxis, skeptophylaxis, specific and nonspecific methods, including the use of tuberculin, milk, sulphur, peptone, etc., and the author's "miasm-free chamber."

The book is of interest to all engaged or interested in the study of allergic diseases and will undoubtedly be read.

*Introduction to Physiologic Chemistry**

IN THIS volume the author has endeavored to present in a coherent manner the main aspects of physiologic chemistry as correlated with recent advances in this subject.

Laboratory methods and tests are omitted, as the purpose of the book is to present a relatively brief, but yet sufficiently comprehensive review of the subject to serve as a source of fundamental principles.

The text is clearly written and evidences an assiduous search of the pertinent literature. As a source of reference the book should prove useful to the student and practitioner, to whom it is addressed.

Elements of Hygiene and Public Health†

A SECOND edition of a well-known English text which has been thoroughly revised. Written particularly to fill the needs of the practitioner at large, the volume well fulfills the purpose for which it is intended.

Manual of Medicine‡

THIS is the third edition, extensively and thoroughly revised, of a useful and comprehensive handbook of medical practice.

Though brief in style, the numerous conditions covered are well described in a clear and practical manner, and the volume should be a useful desk companion.

The Harvey Lectures (1925-1926) §

IN THIS volume are collected seven lectures delivered by different scientists before the Harvey Society in the years 1925 and 1926.

The first (8 pages), by Professor F. R. Nager, of the University of Zurich, discusses various otologic problems and the theoretical and practical results of otohistologic laboratory work. In the second lecture (40 pages), by Dr. J. N. Northrop, is presented a summary of investigations concerned with the dynamics of pepsin and trypsin, the details of the work having been presented previously in the *Journal of General Physiology* (I to VI).

The third lecture (35 pages), by Dr. W. H. Lewis, discusses the transformation of mononuclear blood cells into macrophages, epithelial cells, and giant-cells, and is followed by a comprehensive survey (59 pages) of recent investigations concerned with the parathyroid glands by Dr. J. B. Callys.

Empiricism and rationalism, a discussion of the proper method of treating data from the statistical and mathematical standpoint, is next presented (24 pages) and followed by a lecture (15 pages) upon the historic outline of medical therapy delivered by Professor Knud Faber, of Copenhagen.

The final lecture (14 pages), delivered by Professor Brouwer, of Amsterdam, surveys comparative anatomy and neuropathology.

The volume is without an index, but the typography is excellent.

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*Introduction to Physiologic Chemistry. By M. Badansky, Associate Professor of Physiologic Chemistry, University of Texas. Cloth. Pp. 440, with 40 illustrations. John Wiley & Sons, New York, 1927.

†Elements of Hygiene and Public Health. By Chas. Porter, Lecturer in Public Health, Middlesex Hospital Medical School. Cloth. Pp. 425, with 98 illustrations. Oxford University Press.

‡Manual of Medicine. By A. S. Woodwork, Dean of the Medical School, Westminster Hospital. Third edition. Pp. 523. Cloth. Oxford University Press.

§The Harvey Lectures (1925-1926) Series XXI. Delivered under the auspices of the Harvey Society of New York. Pp. 229. Illustrated. Cloth. Williams and Wilkins Co., Baltimore.

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EDITORIALS

The Nature of Allergens

THE developmental work in anaphylaxis and in allergy has grown to its present status on the basis of the assumption that antigens are protein in nature. Antigens are designated as those substances which, when introduced into the animal body, cause the elaboration of a specific reacting body whose function it is to neutralize or destroy the said substance. The weight of evidence up to the present is that all antigens are proteins. Furthermore, each protein appears to call forth the elaboration of a specific antibody. The specificity of antigens appears to be dependent upon their chemical composition, and is determined by the location of aromatic radicles within the structure of the protein molecule. Free amino acids are not antigenic. Protamins which are complexes of diamino acids are not antigenic. The presence and placement of the aromatic radicles, such as tyrosin, tryptophan, and phenylalanin appear to be at least one factor in determining specificity. Certain chemicals, such as formaldehyde, nitrous acid, and iodine may be introduced into the protein molecule, thereby changing its specificity, that is, making an entirely new protein molecule or antigen.

The purest known proteins act as antigens. Indeed, the purer the protein the more thoroughly it sensitizes the animal and the smaller is the dose necessary. Even crystallized proteins, such as hemoglobin, egg albumen, and edestin are antigenic.

Early efforts were to determine whether anything less than the whole protein molecule would serve as an antigen and, if so, at what stage of its racemization the antigenic power will disappear. Vaughan and his coworkers have maintained that the protein molecule is made up of two factions, a sensitizing, nonpoisonous moiety, and a nonsensitizing, poisonous portion. The sensitizing faction is different in its chemical composition in every protein, while the poisonous faction is the same or nearly the same in all proteins. The nontoxic, sensitizing group appears not to be a biuret body. Zunz, likewise, believes that the amino acid groups responsible for sensitization are not the same as those producing shock. Gay and Robertson concluded that the property of specificity is exercised by certain chemical groups in the protein molecule. Wells and Osborne concluded that specificity is determined by chemical structure rather than by the biologic origin of the protein.

Even the earliest digestion of proteins lessens the antigenic activity. Until recently proteoses and peptones have been considered nonantigenic, but the probability is that proteoses are still antigenic. The tuberculin reaction is probably a proteose sensitization reaction. Gelatin, a hydrolytic protein, is nonantigenic, probably due to its lack of aromatic radicles. Obermyer and Pick have found evidence that the aromatic radicles may be of importance in determining the specific character of immunologic reactions. Wells states that the cleavage products of a protein, even when all are injected together, have no antigenic capacity. When artificially reunited into colloidal molecules (plasteins) their antigenic capacity may be restored. Wells states that in the hydrolytic cleavage of proteins the capacity to invoke antibody formation is lost somewhere between the intact state of the molecule and its complete separation into the constituent amino acids. But as yet we do not know just where. Although there have been many contradictory findings reported as to the antigenic capacity of proteoses, peptones, and peptids, most of the statements have been made with little comprehension of the changes involved in protein hydrolysis or of the nature of the mixtures investigated. The so-called peptones are notoriously variable preparations. To make matters worse, much of the work has been controlled with the anaphylaxis reaction. Here the criteria of reaction are furnished by the behavior of an injected animal, and as many of the anaphylactic symptoms may be essentially reproduced by intoxication with protein cleavage products independent of any immunologic reaction, the value is dubious. A critical investigation and review by Fink emphasizes the worthlessness of much of the published evidence. There is no reason at present to assume that the so-called proteoses or peptones of various designations represent anything but the crudest mixtures, or to expect that constant results will be obtained in any investigation as to their properties.

Abderhalden claims to have produced sensitization with a synthetic polypeptid containing fourteen molecules of leucin and glycin. Zunz claims sim-

ilar results with simpler polymers. These statements are, however, not easily accepted, for the reactions were obtained after intravenous injection into anesthetized rabbits and consisted of fall in blood pressure, increased rate of respiration, and expulsion of feces and urine. These reactions, slight as they were, were not constant. Many of the reactions were quite negative. The plasteins, previously referred to, are, on the other hand, true synthesized proteins produced from proteoses through the reverse action of proteolytic enzymes.

Numerous attempts have been made to produce anaphylaxis to nonprotein substances. This has never been conclusively demonstrated in animals. Early claims of anaphylactic shock to nonprotein substances have usually been discredited and found due to anaphylactoid reactions; thus, lipoids and agar have produced symptoms very much resembling acute anaphylaxis but due to blood changes, one of the most prominent of which consists of thromboses in the pulmonary circulation. However, this work has been carried out chiefly on experimental animals, and it is well known that different animals show the greatest variation of susceptibility.

Pick has found an apparently nonprotein substance in young typhoid cultures which, on injection, produces precipitins but no antibodies. Parker has found a similar substance in pneumococci, influenza bacilli, and staphylococci. Heidelberger and Avery have isolated a polysaccharide from the pneumococcus which causes the production of precipitin. They have found three polysaccharides, at least one of which is entirely nonnitrogenous.

Recently the question has again been raised whether the antigen in some cases of allergy is protein in nature.

There can be no doubt that pollens contain protein substances. Haeyl has obtained an albumin, a proteose, and a glutelin from the pollen of ragweed. These proteins are, however, very feebly antigenic. Ulrich and Koessler have reported successful sensitization of guinea pigs with pollens, but Cook, Coca, and others were unable to sensitize guinea pigs.

Grove and Coca have observed most interesting reactions after tryptic digestion and dialysis of pollen extract. By this method the pollen protein apparently was completely digested and eliminated from the extract and yet there was no demonstrable loss of atopic activity in the treated extract as measured by the skin test. Similar results were likewise obtained with house-dust extract. They conclude that the removal of all protein and other detectable nitrogenous substances from pollen and house-dust extract by means of tryptic digestion and dialysis causes no appreciable lessening of the atopic activity of the extracts. They state that these atopens seem, therefore, not to be proteins in the usual sense of the term.

Tryptic digestion of the proteins of horse dander and of green pea on the other hand, caused a diminution of atopic activity corresponding with the loss of protein by the digestion. These two atopens, therefore, appear to be of protein nature.

Black and Moore have reported similar results, finding no diminution in potency of digested ragweed-pollen extracts, tested by nasal instillation on sensitive subjects.

They further tested 1 per cent and 5 per cent concentrations of ragweed-pollen extract. The stronger contained only three times as much nitrogen but was actually ten times as potent when checked by nasal testing. The conclusion was that the active substance of pollen is not protein, that standardization by nitrogen content and by complement fixation does not determine the content of the active substance, and that treatment with protein-free extract should be effective and at the same time free from the possible objectionable features of injection of extraneous protein. They found that the components which act as antigen when pollen extracts are used in complement-fixation tests are removed by protein digestion, but that the atopic activity was undiminished.

These authors in 1925 treated sixty-two ragweed sensitive persons with protein-free extracts. These extracts gave negative biuret, Adamkiewicz, xanthroproteic and reduced sulphur reactions for protein. Eighty-three and nine-tenths per cent of the series were practically free from symptoms.

Black points out that standardization of pollen extracts by nitrogen content is not reliable, for there may be some proteins present, which are not antigenic. Complement fixation as applied to pollen extracts is only another method of determining the protein in the extract, and while it may serve to distinguish some proteins from others, it is entirely valueless as a means of estimating the amount of active substance.

Grove and Coca suggested that the active substance resembled an enzyme. Black has shaken ragweed extract for four hours in a mechanical shaker and found its activity unchanged. It has been frozen solid for six days and lost none of its activity. Boiling for ten minutes and heating in the autoclave at ten pounds for ten minutes did not affect it. The evidence does not support the assumption that the active substance is an enzyme. The former authors had previously reached the same conclusion.

Black believes that anaphylactic reactions may be secured in guinea pigs by the use of extracts rich in protein and poor in active substance but that these reactions, like complement fixation, are reactions to the protein of the extract and have no relation to the atopen or allergen.

The observations of Grove and Coca and of Black are of the utmost interest in that they again raise the question as to whether substances which produce clinical allergy or anaphylaxis need necessarily be protein in nature. Naturally, the work requires confirmation and study from various additional angles. The question arises whether the nitrogen-free extracts obtained after tryptic digestion were tested against nonsensitive controls to make certain that the subsequent reactions were not nonspecific in character. Presumably they are not, and presumably these control tests have been made.

We should bear in mind that Bernton and his coworkers have found protein derivatives in pollen which appear to be active in extremely minute amounts. One gram of timothy pollen yielded 0.0031 grams of proteose A, 0.0039 of proteose B, 0.0012 of albumin, and 0.0023 grams of glutelin. Fifteen per cent of Bernton's cases were found sensitive to albumin only, 21 per cent to proteose A only, 21 per cent to proteose A and albumin, and 42 per cent to proteose A and B and albumin.

The minute amounts of these substances used in therapy is indicated by the fact that in from sixteen to nineteen routine injections of whole timothy pollen running up to 1600 pollen units, a patient received 0.000034503 grams of proteose A, 0.000043407 of proteose B, and 0.000013356 grams of albumin.

Bernton, Jones, and Csonka assumed that these minute substances are albumin and proteose. Others might take exception to this, claiming that there is so insignificant an amount that some other active constituent, such as that hypothesized by Grove and Coca and by Black, must be present.

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—*W. T. V.*